



**5TH OF DECEMBER
2022**

**ANNUAL
CONFERENCE**

BOOKLET

The organizing committee is pleased by the participation level to the scientific day 2022 of the Département Sciences Biologiques et Médicales. The venue opens the opportunity for creating and strengthening collaborative projects and interactions between the different research laboratories, particularly through selected oral presentations representative of the many research axes developed in our department. Many posters covering diverse scientific areas and platform activities have been submitted and dedicated poster sessions will be held after lunch.

The committee emphasizes this year efforts related to sustainable development through specific action and dedicated talks.

The organizing committee thanks Candice Menegon and Alexandra Prevot, as well as the “scientific animation” committee of the department for all help and support they provided.

Finally a special thanks to Maria Hondele who kindly accepted to be our plenary speaker.

Lucie Brisson, Mélody Dufossée, Nicolas Larmonier, Emilie Montembault, Maya Moubarak, Mathilde Pinault, Fabienne Rayne, Jean-Baptiste Vergnes

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PROGRAM

8H30-8H55 : WELCOME AND REGISTRATION

8H55-9H00 : INTRODUCTION

Alain-Pierre Gadeau

9H00-9H15 : SBM DEPARTMENT PRESENTATION

Katia Boniface

9H15-10H05: KEYNOTE

Chairwoman: Fabienne Rayne

- Pr Maria Hondelé (Biozentrum, Université de Basel, Suisse)
« *DEAD-box ATPases are global regulators of RNA-containing membrane less organelles* »

10H05-10H35 : COFFEE BREAK

10H35-11H20 : SCIENTIFIC SESSION #1

Chairwoman/chairman: Muriel Priault/Arnaud Mourier

- Jenny Wu (IBGC)
- Julien Van-Gils (MRGM)
- Eugenia Basyuk (MFP)

11H20-11H40 : FLASH POSTER PRESENTATIONS

Chairwoman: Lydia Dif

- Laure Migayron (ImmunoConcept)
«*Looking at the memory response in normal appearing skin of vitiligo*»
- Anissa Zaafour (BRIC)
«*Effect of proprotein convertases inhibition on Cancer Stem Cells tumorigenic and invasive properties in gastric adenocarcinoma*»
- Jean-Baptiste Vergnes (MFP)
«*A novel role for the Adenovirus protease during virus entry*»
- Camille Charles (IBGC)
«*Investigation in yeast of the mitochondrial DNA mutation m.9035T>C detected in patients suffering from neuromuscular diseases*»
- Geoffrey Garcia (BMC)
«*NETs/DNAases balance in severe COVID-19 disease*»
- Mélanie Moreau (BRIC)
«*Involvement of Reptin in invadosome formation and tumor invasion*»

11H40-12H25 : SCIENTIFIC SESSION #2

Chairman / Chairwoman: Thomas Daubon/Amélie Guitart

- Akandé Rouchidane-Eyitayo (IBGC)
«*Mechanisms of insertion and oligomerisation of the pro-apoptotic protein Bax in reconstituted membrane systems*»
- Tiffanie Chouleur (BRIC)
«*Multi-parametric analysis of IDH-mutant Glioma progression*»

- Omar Soukarieh (BMC)

«Novel uAUG Creating Variants in the 5'UTR of ENG causing Hereditary Hemorrhagic Telangiectasia»

12H25-12H30 : CASDEN PRESENTATION

Isabelle Lefebvre

12H30-14H20 : LUNCH BREAK

13H20-14H20 : POSTER SESSION (13h20-13h50 odd numbers et 13H50-14H20 pair numbers)

14H20-15H05 : SCIENTIFIC SESSION #3

Chairmen: Manuel Rojo/François Moisan

- Vincent Michaud (MRGM)

«Unsuspected consequences of synonymous or missense variants in OCA2 can be detected in blood samples»

- Guillaume Beucher (MFP)

«Histone chaperones involvement in the spatiotemporal organization of the adenoviral genome chromatin and the viral replication compartment morphology formed by liquid-liquid phase separation (LLPS) in the infected nucleus»

- Domitille Chalopin-Fillot (ImmunoConcept/CBIB)

«Identification of TREM1+ CD163+ myeloid cells as entropic immunosuppressive cells that associate with poor survival»

15H05-15H45 : SUSTAINABLE DEVELOPMENT SESSION

Chairwoman: Bénédicte Salin

- Aurélie Bugeau (LaBri Bordeaux) and Annabelle Collin (INRIA Bordeaux)

«Mesurer et réduire l'impact environnemental du numérique dans l'enseignement supérieur et la recherche»

15H45-16H15 : COFFEE BREAK

16H15-17H00 : SCIENTIFIC SESSION #4

Chairwoman/chairman: Katia Boniface/Jean Rosenbaum

- Fridolin Gross (ImmunoConcept)

- Claire Peghaire (BMC)

- Thomas Mathivet (BRIC)

17H00-17H10 : AWARD WINNERS

17H10 : CLOSING CONCLUSION

KEYNOTE

Pr Maria Hondelé (*Biozentrum, Université de Basel, Suisse*)

«DEAD-box ATPases are global regulators of RNA-containing membrane less organelles»

The ability of proteins and nucleic acids to form biomolecular condensates has recently emerged as an important molecular principle of how cells rapidly and reversibly compartmentalise their components into membrane-less organelles such as the nucleolus, processing bodies or stress granules. How the assembly and turnover of these organelles are controlled, and how these biological condensates selectively recruit or release components are poorly understood. We found that members of the large and highly abundant family of RNA-dependent DEAD-box ATPases (DDXs) are global regulators of RNA-containing phase-separated organelles in prokaryotes and eukaryotes. Using in vitro reconstitution and in vivo experiments, we demonstrate that many DDXs form condensates, with ATP hydrolysis inducing compartment turnover and release of RNA molecules. This mechanism of membrane-less organelle regulation reveals a principle of cellular organization that is conserved from bacteria to humans. Furthermore, we show that DDXs control RNA flux into and out of membrane-less organelles, and thus propose that a cellular network of dynamic, DDX-controlled compartments establishes biochemical reaction centres that provide cells with spatial and temporal control of various RNA-processing steps, which could regulate the composition and fate of ribonucleoprotein particles.

Reference: DEAD-box ATPases are global regulators of phase-separated organelles (2019), <http://doi.org/10.1038/s41586-019-1502-y>

SCIENTIFIC SESSION #1

Jenny Wu (IBGC)

«*The organization of DNA replication: beyond genome duplication*»

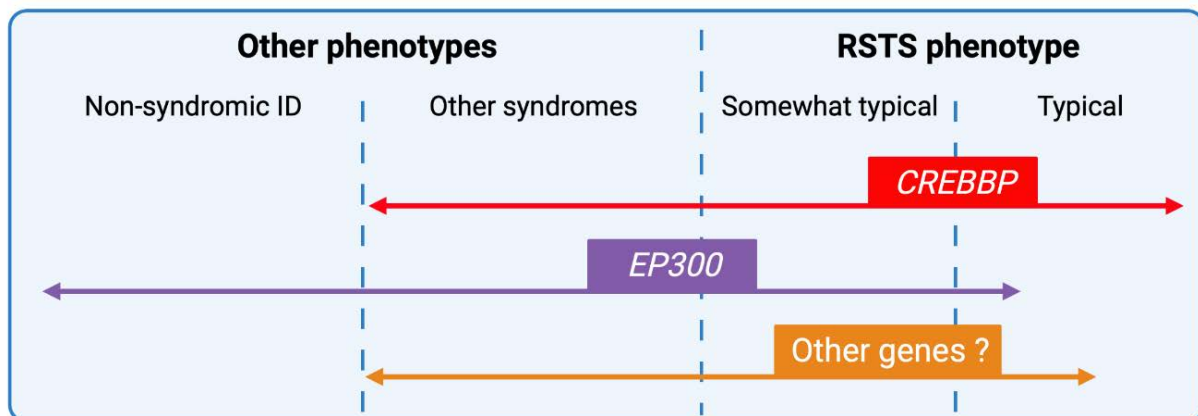
The maintenance of genome integrity is critical for cell growth and proliferation as well as for development and differentiation. Cells employ conserved pathways to ensure the accurate duplication and transmission of genetic information, including multi-layered regulation of DNA synthesis as well as checkpoint systems that verify the completion of replication prior to mitosis. Consistent with the crucial roles of these processes, errors in DNA replication and defects in checkpoint control have been linked to a number of human diseases. In addition, changes in the temporal and spatial organization of genome duplication, which give rise to distinctive profiles of replication initiation along the chromosomes, occur during development and in pathologies. However, the mechanisms that determine the replication program, as well as the biological importance of this pattern, remain elusive. The research in my team addresses these questions, combining a multidisciplinary approach with unique models that we have developed in the fission yeast *Schizosaccharomyces pombe*.

Julien Van Gils (MRGM)

«*Diagnosis of Rubinstein-Taybi syndrome : Identification of the acetylation profiles as epigenetic markers for assessing causality of CREBBP variants GENEPI study*»

The Rubinstein-Taybi syndrome (RSTS) is a rare congenital developmental disorder characterized by a typical facial dysmorphism, distal limb abnormalities, intellectual disability, and many additional phenotypical features. It occurs at between 1/100,000 and 1/125,000 births. Two genes are currently known to cause RSTS, *CREBBP* and *EP300*, mutated in around 55% and 8% of clinically diagnosed cases, respectively. To date, 500 pathogenic variants have been reported for the *CREBBP* gene and 118 for *EP300*. These two genes encode paralogs acting as lysine acetyltransferase involved in transcriptional regulation and chromatin remodeling with a key role in neuronal plasticity and cognition. Because of the clinical heterogeneity of this syndrome ranging from the typical clinical diagnosis to features overlapping with other Mendelian disorders of the epigenetic machinery, phenotype/genotype correlations remain difficult to establish. In this context, the definition of a specific DNA methylation epigraph and more specifically, our study of acetylation and transcriptomic profiles on the iPSC-derived-neurons model will allow the deciphering of the patho-physiological process underlying this

disease and improve the diagnostic efficiency but also open novel therapeutic perspectives. These data highlight the epigenetic regulation of RSTS as a model of chromatinopathy.



Eugenia Basyuk (MFP UMR 5234)

«HIV-1 promoter bursting in T-lymphocytes»

Transcription is discontinuous and alternates between bursts of activity and inactive periods. These stochastic fluctuations of transcription were revealed by live cell imaging as a predominant mode of gene expression in different organisms. They are caused by switching of promoters between active and inactive states. In case of the HIV-1, the fluctuations of transcriptional activity play a key role in a transition between acute infection and viral latency, providing a basis for HIV-1 persistence. We have previously used imaging approaches and analysis in live cells to fully capture transcriptional dynamics of HIV-1 integrated in Hela cells. The results showed that viral RNAs are produced in bursts. These bursts of RNA synthesis are achieved by polymerase convoys: groups of closely spaced polymerases that initiate rapidly one after another and transcribe together through the gene. We also found, that the viral promoter has a probability to enter deeply inactive state, which can last for hours. This inactive state is regulated by promoter proximal pausing of RNA Polymerase II. From the point of view of viral pathogenesis, a generation of polymerase convoys provides a molecular basis for latency exit, while long inactive states are likely to be important for latency entry, since they deplete cells from viral RNAs and decrease the level of virally encoded transcriptional

activator Tat. We are currently investigating if similar mechanisms are at play in T lymphocytes, which are the natural targets of HIV-1. To this end we have established a system to follow transcriptional dynamics of HIV-1 reporters in T-lymphocyte cell line Jurkat. Our approach is based on the bacteriophage MS2 Coat Protein (MCP) fluorescent fusion and an HIV-1 reporter containing 128 MCP binding sites, allowing to follow viral transcription with single molecule sensitivity. We have found that in T-lymphocytes viral RNAs are produced in bursts. The bursting behavior depends on the chromatin environment of the viral integration sites. The role of RNA Polymerase II promoter proximal pausing in regulation of promoter bursting is under investigation.

FLASH POSTER PRESENTATIONS

Laure Migayron (*ImmunoConcept*)

«Looking at the memory response in normal appearing skin of vitiligo»

Resident memory T cells (TRM) are a critical component of the tissue immune response but are also implicated in the physiopathology of cutaneous chronic inflammatory diseases, such as vitiligo. In this depigmenting disease characterized by the loss of epidermal melanocytes, the involvement of skin TRM with a type-1 skewed immune response (production of IFN γ and TNF α) in lesions is commonly admitted. Our recent data suggest an additional role of a type-2 immune response with local production of IL-13. In addition, increasing evidence suggest that non-lesional (NL) skin in cutaneous inflammatory diseases like psoriasis or atopic dermatitis already display an altered immune response. Yet, little is known regarding the T cell immune response NL skin of vitiligo patients. We aimed to decipher the phenotype and function of the TRM infiltrating normal appearing skin of vitiligo patients. While infiltration of CD8 T cells was more prominent in vitiligo perilesional (PL) skin, NL and PL skin displayed similar subsets of TRM defined by the markers CD69, CD103 and CD49a. However, skin T cells isolated from PL vitiligo skin showed a decreased expression of PD-1 which may be responsible of a breakdown of a quiescent state of TRM in NL areas. Interestingly, following ex vivo T cell activation, NL skin displayed an intermediate inflammatory transcriptional profile compared to healthy skin and PL vitiligo skin. In addition, activated NL vitiligo skin T cells produced both type-1 and type-2 related cytokines (including IL-13), albeit at lower levels than activated vitiligo PL skin T cells. Interestingly, IL-13 was implicated in the inflammatory response of epidermal cells by inducing CCL18 production. Taken together, our results suggest that “pathogenic” TRM are already present in pigmented areas in a quiescent state and could contribute to inflammatory flare-ups and relapse of vitiligo.

Anissa Zaafour (*BRIC*)

«Effect of proprotein convertases inhibition on Cancer Stem Cells tumorigenic and invasive properties in gastric adenocarcinoma»

Proprotein convertases (PCs) are enzymes involved in the maturation of numerous precursor proteins implicated in fundamental cellular processes: proliferation, survival, adhesion, invasion, immunity... The role of PCs in tumorigenesis has been extensively studied: it contributes to tumor progression in many malignancies such as rhabdomyosarcoma, colon carcinoma, and others. However, the involvement of PCs in gastric adenocarcinoma (GC) tumorigenesis has been poorly studied until now and need to be investigated. GC is the fourth leading cause of cancer-related death worldwide, it's very

often detected at an advanced stage and associated with poor prognosis and high risk of relapse. This can be explained by the presence of cancer stem cells (CSCs), a subpopulation of cancer cells able to self-renew, differentiate, initiate tumor growth, metastasize, resist to conventional therapies, and trigger cancer relapse. CSCs hold their properties and survival through hijacked signalling pathways such as the Hippo pathway as it has been demonstrated in GC. They possess an epithelial to mesenchymal transition (EMT) signature reflecting cancer aggressiveness. Use of decanoyl-RVKR-chloromethylketone (CMK), general chemical PCs inhibitor, in four different GC cell lines, allowed to highlight that PCs inhibition decreased GC CSCs ability to initiate tumorspheres, sustain tumorspheres growth, as well as their drug efflux capacities. It also reduced the transcriptional activity of downstream YAP/TAZ/TEAD oncogenic effectors of the Hippo pathway, suggesting CMK could inhibit CSCs properties via YAP/TAZ/TEAD activity. Moreover, the invasiveness of GC cell lines was highly impaired by PCs inhibition. This effect was associated to a decrease of expression of some invasive and mesenchymal markers and of EMT transcription factors nuclear expression: ZEB1 and Snail. To conclude, PCs inhibition seems to be a potential strategy to target CSCs in GC. Further investigations are required to refine this strategy and better understand the molecular mechanisms implicated in anti-CSC effects in GC.

Jean-Baptiste Vergnes (MFP)

«A novel role for the Adenovirus protease during virus entry»

Adenoviruses are non-enveloped viruses that enter the cell by endocytosis and escape from the endosomal compartment. To cross the endosomal membrane the capsid undergoes partial disassembly and releases capsid protein VI inside the endosome. Protein VI, with its amphipathic helix, binds and damages membranes. Cytosolic virion will then be transported to the nucleus to initiate viral gene expression and replication. It remains unclear how the partial disassembly allows protein VI release. Possible mechanisms include acidification inside the endosome and/or mechanical disruption induced by the binding of the virion to cell receptors. The Adenovirus protease, a sequence-independent DNA binding protein incorporated inside the virion, is processing different proteins of the Adenovirus through a one dimensional biochemical reaction. This step called maturation is happening after the virus assembly and is essential for the virus infectivity. Indeed, a mutant with unprocessed proteins remains stable inside the endosome, thus there is no release of protein VI and no membrane damages. Consequently, with unprocessed proteins, the Adenovirus is unable to escape the endosome. We produced and purified the Adenovirus protease in order to reconstitute the maturation process in vitro to better understand its role in the viral cycle. We observed an unexpected cleavage that could be a trigger in the virus partial disassembly. Our data thus

indicate that the protease may have so far unrecognised functions during virus entry steps.

Camille Charles (IBGC)

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

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.

Geoffrey Garcia (BMC)

«NETs/DNases balance in severe COVID-19 disease»

Neutrophils Extracellular Traps (NETs) are web-like structure composed of DNA and proteins from neutrophil cytoplasm. They are released in a process called NETosis from neutrophil to trap pathogens in a process called immunothrombosis: NETs, with other immunological actors, activate coagulation to form a clot containing the pathogen, thus preventing its

dissemination in the organism. Physiologically, they are regulated by DNases. Interestingly, An increase of NETs markers has been observed during COVID-19: excess of NETs contribute to the physiopathology of severe COVID-19 by an uncontrolled immunothrombosis and coagulopathy. We hypothesised that a decrease of DNases activity in severe COVID-19 patients could be responsible of a NETs clearance impairment, and lead to disease progression. We developed a method to explore total DNases activity in human samples and evaluated it on 34 patients with moderate and severe COVID-19 included in COLCOV19 protocol. We measured two NETosis markers: citrullinated histone H3 (H3cit) and DNA-H3cit complex. We found that our fluorimetric approach to explore total DNases activity in human sample is an easy, repeatable and reproducible method, and we define pre-analytical conditions to perform it. Our results showed that patients with severe symptoms have an increase of NETosis markers compared to mild patients, as described in the literature. In addition, total DNases activity is increased in severe patients. However, NETosis markers/total DNases activity ratio is increased in severe patients, showing an unbalanced regulation of NETs. We believe that DNases activity is not increased enough to limit NET elevation in human plasma during severe COVID-19 and lead to an aggravation of the disease by an impairment of NETs clearance. Our work confirms the importance of NETosis and DNases implication during severe COVID-19 and leads to explore and understand the mechanism of DNases dysregulation in this disease.

Mélanie Moreau (BRIC)

«Involvement of Reptin in invadosome formation and tumor invasion»

Metastasis formation is the main cause of cancer related death. They are the consequence of tumor invasion that is the ability of cancer cells to colonize new tissue. To do so, cells must migrate across anatomical barriers, notably by degrading the extracellular matrix (ECM). This ability is conferred by invadosomes, which are membrane protrusions composed of F-actin structures associated with MMPs activity. In a previous study, we used an approach combining laser microdissection and mass spectrometry analysis to define the invadosome proteome in the NiH3T3-SrcY527F cell model. These cells overexpress a constitutively active form of Src protein promoting rosette invadosome formation. This approach revealed that Reptin is 6 times enriched in invadosomes in comparison with the total cell lysate. Reptin is a AAA+-ATPase involved in different cellular functions including DNA repair, replication and molecular co-chaperoning complexes. Reptin is a member of the R2TP complex, which is required for the assembly and conformation of many protein complexes. We demonstrated that Reptin, as well as the other members of the R2TP complex (Pontin, RPAP3 and PiH1D1), co-localize with rosette invadosomes. By a siRNA approach we have shown that Reptin depletion

significantly decrease the NiH3T3-SrcY527F ability to form invadosomes and to degrade the ECM. Moreover, in Reptin depleted cells, we noticed a recover of the wildtype phenotype characterized by the presence of stress fibers and the absence of invadosomes. That point reflects a loss of SrcY527F activity suggesting a molecular link between Src and Reptin. We confirmed this hypothesis by showing a Reptin and Src co-localization and by highlighting a decrease of Src-Tyr419 phosphorylation state in Reptin depleted cells without affecting its total expression level. To identify the molecular mechanism involved in the modulation of Reptin-dependent Src activity, we use bibliographic and exploratory proteomic approaches to determine proteomic profiles with or without Reptin and Reptin partners and their major functions in invadosomes. Therefore, we revealed the involvement of autophagy in the regulation of proteins, such as Fak, in a Reptin-dependent manner. Taken together these results will allow a better understanding in the mechanism of invadosome formation mediated by Reptin and the R2TP complex. This work will allow a better comprehension of mechanisms involved in the process of tumor invasion.

SCIENTIFIC SESSION #2

Akandé Rouchidane-Eyitayo (IBGC)

«Mechanisms of insertion and oligomerisation of the pro-apoptotic protein Bax in reconstituted membrane systems»

Apoptosis is the process by which animal cells initiate self-destruction in response to a death signal (e.g. DNA damage). The intrinsic pathway of apoptosis is controlled by proteins of the Bcl-2 family. Among them, the pro-apoptotic multidomain protein Bax plays a central role in this process. Bax is present in an inactive conformation in the vast majority of non-apoptotic cells. After an apoptotic signal, Bax is activated and relocated to the outer mitochondrial membrane (OMM), where it is oligomerised to form a large pore. The formation of this pore promotes the release of several so-called apoptogenic factors, which contribute to the downstream activation of caspases, and other pro-apoptotic factors. In solution, Bax is organised into 9 α -helices. In its soluble conformation, the hydrophobic 9 α helix is stabilised in a hydrophobic groove formed by the 3 BH domains of the protein. The process of insertion and oligomerisation of Bax into MOM remains poorly understood. Unlike its anti-apoptotic partners (Bcl-2 and Bcl-xL) which have an anchoring sequence in the MOM in their c-terminus, this same domain in Bax is important for maintaining the soluble form of the protein (Kaufmann et al 2003)[1]. It is therefore important to understand how Bax selectively permeabilises the MOM when apoptosis is induced. Bellot et al (2007)[2] showed that when apoptosis is induced in glioblastoma cells, the insertion of Bax into the MOM is dependent on the presence of TOM22 (a subunit of the mitochondrial protein import complex TOM). However, the mechanism remains unclear. In this work, we reconstructed in an in vitro production system the activation and insertion of Bax into a membrane environment (nanodiscs) (Rouchidane et al 2022)[3] via its interaction with the TOM22 subunit. Our results show that when Bax is produced in vitro in the presence of nanodiscs, its spontaneous insertion into the nanodiscs is greater when co-produced with TOM22. We also observed this phenomenon with another mitochondrial localisation protein and partner of Bax which is the anti-apoptotic protein Bcl-xL. Furthermore our results suggest that the interaction between Bax and Bcl-xL is transient in solution and that insertion of the Bax-Bcl-xL heterodimer into a membrane environment via the c-terminal helix of Bcl-xL is important to stabilise the heterodimer.

Tiffany Chouleur (BRIC)

«Multi-parametric analysis of IDH-mutant Glioma progression»

Isocitrate dehydrogenase (IDH) mutant gliomas remain lethal brain cancers which impair quality of life in young adults. These tumors are molecularly and cellularly heterogeneous and have a wide range of survival prognoses. As a consequence, the identification of patients at risk of early recurrence remains

an unmet need. Here, we analyzed imaging, transcriptomic and proteomic profiling using machine learning to 1) describe biological characterization of different subtypes of IDH-mutant gliomas categorized by PET and histology, 2) reinforce the integration of PET metrics in the classification of IDH-mutant gliomas, 3) improve the patient stratification with novel signatures of patient risk of recurrence based gene expression, protein level and/or imaging. Our integrative analysis provides a better stratification of IDH-mutant gliomas patients and their risk of recurrence, which will lead to a better monitoring of the clinical evolution of the disease.

Omar Soukarieh (BMC)

«Novel uAUG Creating Variants in the 5'UTR of ENG causing Hereditary Hemorrhagic Telangiectasia»

Hereditary haemorrhagic telangiectasia (HHT) is a rare disease characterized by epistaxis, mucocutaneous telangiectasia and arteriovenous malformations affecting multiple organs. Severe complications can be fatal in 10% of cases. About 80% of patients carry rare coding mutations in ACVRL1, ENG or SMAD4. We here report the identification of 2 new variations, c.-79C>T and c.-68G>A, in the 5'UTR of ENG in 2 unrelated patients. They are predicted to create upstream AUGs (uAUGs) in the 5'UTR in frame with the same stop codon located within the coding sequence, generating Overlapping Open Reading Frames (uoORFs). In order to assess the potential effect of these variants on ENG, we performed functional assays based on the expression of wild-type and mutant constructs in human cells. We found that they were associated with a decrease of protein levels (HeLa and HUVECs), and luciferase activity. Very interestingly, these variants reduce the BRE activity in response to BMP9 in vitro. This assay, applied on 5'UTR variants for the first time in this study, is essential for ENG variants before providing a definitive molecular diagnosis. We applied the same experimental workflow on additional uoORF-creating variants (c.-142A>T, c.-127C>T and c.-10C>T) in the 5'UTR of ENG reported in HHT patients without extensive functional investigations, and found similar results. Additional experiments relying on artificial deletions in our mutated constructs suggested that created uAUGs could initiate the translation and that the associated effect is caused by an alteration of the translation mechanism. In order to confirm these data, we are performing translation-specific assays in collaboration with the Hashem lab at the IECB (Institut Européen de Chimie et Biologie). Overall, we here identified two new 5'UTR ENG variations in HHT patients and highlighted the regulatory role of uoORFs. That contributes to ameliorate molecular diagnosis in HHT and to open perspectives for new therapeutic approaches.

SCIENTIFIC SESSION #3

Vincent Michaud (MRGM)

«Unsuspected consequences of synonymous or missense variants in OCA2 can be detected in blood samples»

Oculocutaneous albinism type 2 (OCA2) is the second most frequent form of albinism and represents about 30% of OCA worldwide. Like for other OCA types, patients present with hypopigmentation of hair and skin as well as severe visual abnormalities. We focused on a subgroup of patients with no confirmed genetic diagnosis because at least one of their identified variants in OCA2 is of uncertain significance (VUS). These VUS, for which in-silico prediction tools are poorly effective, are mainly missense, synonymous or intronic. We first selected 29 patients bearing rare variants lying in or around exon 10, a sequence highly sensitive to alternative splicing. We investigated the effect of each of these variants on exon 10 skipping in a quantitative way. By minigene assay, we show that both intronic and synonymous VUS of exon 10 can result in enhanced exon skipping to an extent that supports pathogenicity. Unlike TYR (OCA1), OCA2 expression is not restricted to pigment cells. We therefore tested the ability to detect OCA2 transcripts on mRNA extracted from blood collected in PAXgene tubes. We first show that excessive skipping of exon 10 can be detected directly on such samples. We next found that blood OCA2 mRNA analysis also enables the identification of variants that impair transcription or mRNA stability. For instance, the most common OCA2 pathogenic variant c.1327G>A, predicted as missense (p.Val443Ile), is unexpectedly shown to result in the absence of detectable OCA2 mRNA in blood cells. Confirming such an effect in skin melanocytes of patients carrying this allele will allow us to reconsider the way this variant causes the pathogenic loss of function of OCA2. All in all, we have developed a new simple and direct method to test the potential effect of OCA2 variants on transcription products. This should help to elucidate yet unsolved OCA2 patients and to improve genetic counseling.

Guillaume Beucher (MFP)

«Histone chaperones involvement in the spatiotemporal organization of the adenoviral genome chromatin and the viral replication compartment morphology formed by liquid-liquid phase separation (LLPS) in the infected nucleus»

Adenovirus is a respiratory virus belonging to the family of double stranded DNA viruses. It's genome is highly compact inside the viral particle and organized into chromatin by virtue of the protamine-like protein VII (pVII). Nuclear genome delivery in a target cell is followed by decompaction and partial eviction of pVII and replacement by cellular histones to proceed into transcription and/or replication. At late stages the process is reversed and viral genomes are condensed and packaged into pVII chromatin without

cellular histones. At current few factors involved in driving this reversible chromatinization are known. Likely candidates involve histone chaperones, which are classed into three different groups acting replication dependent, replication independent or following DNA repair. Viral genome replication occurs in virus induced membraneless organelles called viral replication compartment (RC) formed with host factors and viral proteins. RCs are morphologically dynamic and two distinct RCs can be distinguished during early vs. late replication. Early RCs are presumed to replicate genomes for gene expression while late RCs surround bodies called viral induced post replication bodies or ViPRs bodies which are the site of viral genome accumulation likely for viral production. We try to elucidate the mechanism driving RC and ViPRs formation and what allows the recruitment or exclusion of both host and viral proteins. We think that the mechanisms involved are likely liquid-liquid phase separation (LLPS). We first focused on identifying histone chaperones involved in the spatiotemporal organization of the infected nucleus. We now study if histone chaperones are involved in the RC morphology and its dynamics. We use fluorescence microscopy and seek functional validation by inhibition and depletion experiments of selected histone chaperone candidates.

Domitille Chalopin-Fillot (*ImmunoConcept/CBIB*)

«Identification of TREM1+ CD163+ myeloid cells as entropic immunosuppressive cells that associate with poor survival»

Hepatocellular carcinoma (HCC) is an inflammation-associated cancer arising from viral and non-viral etiologies. Immune checkpoint blockade primarily benefits patients with viral HCC. Expansion of suppressive myeloid cells is a hallmark of chronic inflammation and cancer, but their heterogeneity in HCC is not fully resolved and might underlie immunotherapy resistance in the steatohepatitis setting. Here, we present a high resolution atlas of hepatic innate immune cells (~100,000 single-cell transcriptomes) from patients with HCC and unravel several discrete populations of entropic monocytes and dendritic cells that expand in the tumoral tissue. Among them, we identified a population of suppressive THBS1-Monocyte that is rare in viral HCC but expands in the steatohepatitis setting. These THBS1-Monocytes dually express granulocyte- and macrophage-lineage genes and are selectively marked by elevated expression of TREM1 and CD163. They most potently suppress T cell activity *ex vivo*, highly express TGF β and are spatially enriched with FAP+ fibroblasts at HCC fibrotic lesions. The density of THBS1-Monocytes significantly correlates with advanced grades HCC and poor patient survival, and associates with resistance to immune checkpoint blockade in other solid tumors. The expression of TREM1 alone confers poor prognosis in HCC and blockade of its signaling in mice promotes tumor eradication. Our data support myeloid subset-targeted immunotherapies via TREM1 to treat HCC.

SCIENTIFIC SESSION #4

Fridolin Gross (*ImmunoConcept*)

«What is a cell type?»

Classifications are fundamental conceptual tools for the systematization of scientific knowledge. Some classification systems, such as the periodic table of elements, are thought to reflect the structure of the natural world rather than just the interests of human beings and result in so-called «natural kinds». Philosophers have debated whether natural kinds also exist in biology, generally considering classifications of organisms into species as the main example. Cell types have not received much attention in this context, although they are arguably also fundamental to many areas of biology. Many biologists seem to tacitly assume that different criteria coincide and result in an objective classification of cell types. However, recent research suggests a more complex picture where cellular phenotypes are much more plastic and heterogeneous than previously assumed, thus rendering the notion of cell type problematic. The aim of this project is thus to clarify the concept of «cell type». In particular, it puts forward two hypotheses: 1) The different criteria, apparently in conflict, can be aligned when considering their conceptual relationships, suggesting a monistic notion of cell types as «functional species». 2) The new techniques do not lead to a conceptual revision of this notion, but rather to a revision of factual knowledge about cell types.

Claire Peghaire (*U1034 BMC*)

«The endothelial ubiquitine ligase TRIM47 : a new contributor to cerebral small vessel disease ?»

Cerebral small vessel disease (cSVD) is a leading cause of stroke and a major contributor to cognitive decline and dementia. It is a chronic, progressive vascular disease that is diagnosed by lacunes and white matter hyperintensities in MRI. cSVD is multifactorial (aging, oxidative stress, diabetes, hypertension, genetic factors) and characterized by disruption of the blood-brain barrier (BBB). The U1034 laboratory aims to improve knowledge about the endothelium, to use it as a potential marker of cSVD and treat its dysfunction. A collaboration with clinicians allowed us to identify (exome sequencing) in a cohort of patients with cSVD the presence of a nonsense mutation on the TRIM47 gene, encoding for an ubiquitin ligase protein E3 strongly expressed by endothelial cells. Our current project combining complementary in vivo and in vitro models aims to study the role of TRIM47 in brain physiology, the maintenance of BBB homeostasis and its potential contribution to the development of cSVD.

Thomas Mathivet (BRIC)

«*Window on neurovascular pathologies*»

Neurovascular pathologies, such as brain tumor (glioma), stroke, or traumatic brain injury are accompanied by alteration of blood vessels, causing a defect in their perfusion, but also leakage leading to oedema formation or hemorrhages. In order to dissect the mechanisms at the origin of this vascular damages in order to restore the function of cerebral blood vessels, we study the dynamics of these pathologies by high-resolution intra-vital imaging through cranial windows.

These studies have enabled us to identify the responsibility of our innate immunity (macrophages) in these phenomena.

POSTER SESSION

P1 - Disulfiram, a promising drug candidate for the treatment of Barth Syndrome and possibly other mitochondrial diseases

Claire Almyre

Barth syndrome (BTHS) is a rare X-linked cardiomyopathy caused by mutations in a nuclear gene (TAZ) that encodes an acyltransferase (called Taffazine) involved in the maturation of cardiolipin, a phospholipid with multiple functions in mitochondria that optimize the oxidative phosphorylation process (OXPHOS). To aid in the search for drug candidates for the treatment of this disease, we have constructed a yeast strain in which the orthologous gene has been deleted from the chromosome (*taz1Δ*). Similar to BTHS patient cells, *taz1Δ* yeast cells showed defects in the biogenesis of the OXPHOS system and thus exhibited a severely impaired growth from carbonaceous substrates requiring necessarily functional mitochondria to be metabolized. We have isolated several molecules improving this growth -and thus the mitochondrial function- from chemical libraries constituted of compounds at least in phase II of clinical tests and for the most part already used in the human health field. Our approach is therefore a therapeutic repurposing. One of these molecules, disulfiram (DSF), has been used for decades for the treatment of chronic alcoholism. When administered, alcohol intake induces migraines due to the inhibition of acetaldehyde dehydrogenase in the mitochondria. Other well-known inhibitors of this reaction have not shown beneficial effects in *taz1Δ* yeast cells, indicating that the improvement of their mitochondrial function by DSF has another origin. We therefore directed our study towards another well-known property of this molecule: its ability to transport copper. First, we tested other molecules with this property, including elesclomol and pyrithione. Like DSF, these two molecules were found to improve the respiratory growth of the *taz1Δ* mutant. Furthermore, supplemental copper in the culture media alone was also active, indicating that it is indeed its ability to transport copper that allows DSF to correct the mitochondrial defect in the yeast model of Barth's syndrome. Remarkably, we found that DSF is also active in other yeast models of mitochondrial diseases, including diseases associated with perturbations in mitochondrial translation, assembly of complexes III and IV of the OXPHOS system, mitochondrial DNA replication, and mitochondrial dynamics. In addition, DSF has shown (in media promoting respiratory metabolism) significant beneficial effects on the proliferation and survival of human cells with the same defects. These observations make DSF a promising drug candidate with a broad spectrum of action for the treatment of mitochondrial diseases. Therefore, we are testing this molecule in a mouse model of Barth's syndrome and are trying to understand the mechanism by which it compensates for mitochondrial defects causing human diseases.

P2 - 1D continuous gel electrophoresis composition for the separation of deamidated proteins

Axel Boudier

Deamidation is a spontaneous modification of peptides and proteins that has potent repercussions on their activity and stability in vivo and in vitro. Being able to implement easy techniques to detect and quantify protein deamidation is a major goal in this field. Here we focus on electrophoretic methods that can be deployed to assess protein deamidation. We provide an update on the use of Taurine/Glycinate as trailing ions to assist the detection of several examples of deamidated proteins, namely the small GTPases RhoA, Rac1 and Cdc42, but also the oncogene Bcl-xL and calcium-binding Calmodulin. We also report on the use of imidazole as a counter ion to improve the focusing of deamidated bands. Finally, we provide examples of how these gels proved useful to compare on full-length proteins the effect of ions and pH on the catalytic rates of spontaneous deamidation. Taken together, the electrophoretic method introduced here proves useful to screen at once the effect of various conditions of pH, ionic strength and buffer ions on protein stability. Direct applications can be foreseen to tailor buffer formulations to control the stability of proteins drug products.

P3 - Influence of immunosuppressive myeloid cells on cancer stemness promotion in breast cancer

Thomas Boyer

Compelling evidence has indicated that cells of myeloid origin represent major components of the complex immunosuppressive tumor microenvironment. These myeloid cells such as tumor-associated macrophages, neutrophils and so-called «myeloid-derived suppressor cells» (MDSC) among others have been widely described for their immunosuppressive properties and their ability to inhibit anti-tumor immune responses. They thus represent major obstacles for efficient immunotherapeutic approaches. However, beyond this cardinal immunosuppressive function, MDSC are also endowed with a broad array of «non-immunological» tumor-promoting functions. Indeed, accumulating evidences has demonstrated that these cells can directly promote primary tumor cell survival and proliferation and promote local tissue invasion among others. Importantly, MDSC play a key role in the preparation of the pre-metastatic niches before the arrival of cancer cells, thus contributing to the preparation of the «soil» for seeding by metastatic tumor cells. The role of cancer-induced myeloid cells in resistance to chemotherapy and immunotherapy has also been described. Evidence has also emerged that tumor-induced immunosuppressive myeloid cells may impact cancer stem cells (CSC), a subpopulation of cancer cells within the tumor, defined by self-renewal, asymmetrical division and

differentiation properties, giving rise to more or less differentiated cells composing the tumor mass. Using 3-D tumorsphere formation assays we demonstrate that human monocyte-derived suppressor cells (HuMoSC) are endowed with the capability to promote stemness features in breast cancer cells in a contact- dependent manner and these interactions confer to cancer cells chemotherapy resistance properties. Moreover, our data provide insights into the ability of mouse-derived MDSC to increase tumorsphere formation. Finally, we confirmed our results with the study of myeloid cells isolated for breast tumor bearing patients.

P4 - Targeting metabolic vulnerabilities in glioblastoma

Ahmad Charanek

Glioblastoma is the most common and aggressive primary brain cancer, with a median survival of 16 months. A subset of cells within these tumours, termed glioblastoma stem cells (GSCs), displays high tumourigenic capacity and resistance to conventional therapies, and are therefore considered responsible for tumour recurrence. Glioblastomas have been shown to mainly rely on glucose as an energy source. However, a growing body of literature reports that GSCs are less glycolytic than their differentiated progeny and can thrive on oxidative phosphorylation (OXPHOS) to survive, sustain stemness and fuel tumourigenic potential. Thus, targeting OXPHOS could be a promising strategy to halt energy production and starve GSCs to death. However, under metabolic stress, GSCs can also switch to a more glycolytic phenotype, suggesting a metabolic plasticity feature and thus adding a layer of complexity to target GSC metabolism. In the current study, we have investigated the impact of compound X (CX), recently shown to impair mitochondrial respiration, on the fate and tumourigenesis of GSCs. Using multiple patient-derived and murine GSC models, we have established that CX inhibits GSC proliferation, impairs self-renewal and results in downregulation of stemness-related pathways in vitro. Using a syngeneic mouse model of glioblastoma in vivo, we have shown that CX delays tumourigenesis. While a drastic decrease in cell proliferation was observed upon CX treatment, annexin/PI analysis revealed that CX does not induce cell death. This suggests that GSCs gain a slow-cycling dormant phenotype to resist energy depletion. Mechanistically, we have established that CX targets complex I of the mitochondrial respiratory chain leading to an impairment of the mitochondrial OXPHOS. Interestingly, inhibition of OXPHOS by CX was associated with extensive production of extracellular lactate, indicating that, at least, a subset of the dormant GSCs gain metabolic adaptability by reprogramming their metabolism to glycolysis. Thus, to tackle the metabolic plasticity in GSCs, we adopted a new strategy that involves sequestration of the lactic acid intracellularly via disruption of lactate transporters. Treatment of GSCs with a specific monocarboxylate transporter

(MCT) 1/2 inhibitor, AZD3965, or MCT1/4 inhibitor, syrosingopine alone did not significantly impact cell growth. Strikingly, combined CX treatment with either AZD3965 or syrosingopine resulted in high intracellular lactate buildup, increased reactive oxygen species, and led to drastic cell lethality in multiple GSC models. This study revealed that re-routing OXPHOS-dependent GSCs to glycolysis offers a vulnerability point to sensitize GSCs to cell death. Dual inhibition of OXPHOS and lactic acid export could be a promising therapeutic approach to deplete malignant GSCs and suppress glioblastoma tumourigenesis.

P5 - The pblMS mutant presents defects in cortical myosin dynamics and daughter cell size asymmetry during cytokinesis

Marie-Charlotte Claverie

Cytokinesis occurs subsequently to sister chromatid segregation. It requires the assembly of an acto-myosin contractile ring. Myosin activity generates the forces necessary to drive the ingression of the cleavage furrow. The activity of the small GTPase Rho1, localized at the plasma membrane, is essential for this process and determines the position of the contractile ring and its ingression. Rho1 activity is catalyzed by a guanine nucleotide exchange factor called Pebble (Pbl). The mechanism by which Pbl concentrates at the equatorial zone to activate Rho1 is through its interaction with RacGAP50C. The molecular pathway that specifies the zone of Rho1 activation to initiate contractile ring assembly is well defined, but less is known about the mechanism that maintain the contractile ring position while sustaining efficient constriction. This mechanism is critical to preserve the size of the resulting daughter cells, hence is particularly important during asymmetric stem cell division where cell size determines cell fate. *Drosophila* neuroblasts are progenitors of the central nervous system, dividing to generate a larger apical cell that retains the neuroblast fate and a smaller basal Ganglion Mother Cell (GMC). Our team recently identified novel Pbl-dependent myosin dynamics during neuroblast division, called myosin eux. At mid-ring closure, a pool of myosin undergoes outward flow from the contractile ring, invading the entire cortex. Attenuation of Pbl function using the pblMS mutant impairs myosin enrichment at the polar cortex during division. The absence of myosin eux does not affect the rate of furrow ingression but remarkably, a significant proportion of pblMS mutant cell divisions produces abnormally small GMC, associated with decline in locomotion and lifespan. In absence of cortical myosin enrichment, the furrow position cannot be corrected during ring constriction, suggesting the existence of a mechanism sensing and correcting furrow position in WT cells.

P6 - Development of a novel biohybrid polymersome $\gamma\delta$ T-cell conjugated technology in response to Cytomegalovirus infection

Selma Cornillot-Clément

Development of a novel biohybrid polymersome $\gamma\delta$ T-cell conjugated technology in response to Cytomegalovirus infection Selma Cornillot-Clément, Anouk Martin, Matthieu Kamierzac, Emmanuel Ibarbour, Gabriel Marseres, Sébastien Lecommandoux, Julie Déchanet-Merville. Objective: The global objective of this chemistry-immunology interdisciplinary project is to improve drug vectorisation by making use of the migration capacity of immune cells. The pathological model chosen is the infection by Cytomegalovirus (CMV) a life threatening virus in immunosuppressed individuals and in neonates. Our system is based on the design of “hybrid” systems conjugating (i) custom-made functional polymersomes (polymer vesicles) loaded with an antiviral molecule (Ganciclovir) with (ii) $\gamma\delta$ Gamma-Delta T lymphocytes ($\gamma\delta$ T cells) which are able to control Cytomegalovirus virus (CMV) by killing infected cells and expanding in infected tissues. These cells will be used as “Trojan horses” cell therapy with the aim of specifically targeting and accumulating drug-loaded polymersomes in tissues infected by CMV. This strategy aims to minimise the serious side-effects associated with systemic administration of ganciclovir (leukopenia, drug resistance). Among this project, my own objective is to set up $\gamma\delta$ T cells - polymersomes conjugation which relies on Click Reaction between (i) azido-sugars metabolized by $\gamma\delta$ T cells and expressed on cell surface, and (ii) an azide reactive moiety (dibenzocyclooctyne group, DBCO) present on polymersomes. Results: Conclusive tests about the capacity of immune cells to metabolize azido-sugars and to couple with DBCO were done using peripheral mononuclear cells (PBMCs) or clinical-scale expanded $\gamma\delta$ T cells. Click reaction was evaluated using a Fluorescein amidites (FAM)-coupled DBCO allowing quantification by flow cytometry and cellular localisation by confocal microscopy. Kinetics of incubation and concentrations of azido-sugars have been validated. Click reaction on immune cells did not affect their capacity to be activated, as demonstrated by their secretion of IFN γ after incubation with PMA and ionomycin or with agonist anti-CD3. They should therefore, be able to react to infected cells as it will be tested in the next steps Conclusion: Feasibility of immune cell conjugation using click chemistry is validated. Our next objectives are to conjugate $\gamma\delta$ T cells with DBCO-engineered polymersomes and to test the bioactivity of this hybrid system in vitro and in vivo. The proof of concept will be established on the CMV model, but this approach can subsequently be generalized and applied to a range of other diseases implying a major role of lymphocytes including cancer.

P7 - Two RhoGEF isoforms with distinct localizations act in concert to promote robust asymmetric cell division

Irène Deduyer

Cytokinesis is the last phase of the cell division that partitions the cellular content into two daughter cells. It relies on the formation of an acto-myosin contractile ring that generates the forces necessary to form the cleavage furrow. In this process, the activity of the small GTPase Rho1 at the plasma membrane drives the assembly and constriction of the contractile ring. The activation of Rho1 (RhoA) is catalyzed by the RhoGEF Pbl (Ect2). The canonical mechanism that concentrates Pbl at the equatorial region acts through the microtubule-associated protein RacGAP50C (mgcRacGAP), part of the centralspindlin complex. The interaction between RacGAP50C and Pbl releases Pbl auto-inhibition, promoting its accumulation at the cell equator. The molecular pathway specifying the region of active Rho1 is well defined, but less is known about the mechanisms that maintain the contractile ring position while sustaining efficient constriction. Here, using *Drosophila* neuroblasts, cells dividing asymmetrically to generate a larger apical cell that retains the neuroblast fate and a smaller basal Ganglion Mother Cell (GMC), we show that two splicing variants of Pbl with different localizations control asymmetric divisions in this model. Pbl-A, enriched at the furrow, promotes efficient Rho1 activation and therefore ring ingression. In contrast, Pbl-B localized throughout the cortex, stimulates cortical myosin enrichment and broadening of Rho1 activity. Together, those two isoforms act in concert to ensure robust asymmetric cytokinesis.

P8 - 3D cell models to study liver physiopathology : From healthy liver to NASH and HCC disorders

Adèle Delamarre

Liver is a central organ involved in critical functions, among them metabolism, lipid homeostasis or detoxification. Hepatocellular carcinoma (HCC) is the most common liver cancer and a major public health problem worldwide. With an increasing incidence linked to obesity and diabetes, NASH is the fastest growing etiology of HCC and is becoming the leading cause of HCC worldwide. Modeling NASH disease and HCC carcinogenesis is crucial to better understand underlying molecular pathways and find new therapeutic targets. 2D cellular models are easy to manipulate but too distant from the complexity of liver pathophysiology. Existing *in vivo* mouse models of NASH do not recapitulate the whole spectrum of the human pathology. Moreover, we have to think about reducing experimentation on mouse models in accordance with the 3R strategy. 3D cell models have seen a great breakthrough since twenty years to answer this challenge, from monocellular spheroids to complex organ-on-

chip. 3D cell models enable a better modeling of the disease thanks to 3D cell interaction, better cell differentiation and function. Additionally, 3D models are suitable for complex multicellular models and allow to recapitulate a suitable microenvironment. Existing models may recapitulate NASH disease but no 3D model is currently available and easy to manipulate with a fairly long viability to study HCC carcinogenesis on NASH. Our goal is to set up new 3D cell models for each step of the disease progression : from healthy liver to NASH and HCC development to allow functional and molecular investigation of carcinogenesis. We use primary human hepatocytes (PHH) and HepaRG® cell line that are grown either in normal conditions or in a culture medium enriched in fatty acids and LPS in order to induce NASH phenotype. Our 3D cell models are based on two processes : spheroids and cell encapsulation technology in 3D alginate capsules.

P9 - Role of Furin in Colon Cancer Stem Cells Phenotype in KRAS and BRAF-Mutated Colon Tumors

Jean Descarpentrie

Found in respectively 50% and 10% of colorectal cancer (CRC) patients, KRAS and BRAF gene inactivating mutations mediate colon cancer initiation through cancer stem cells (CSCs) activation. CSCs are involved in tumor progression, metastasis induction, chemotherapy resistance, and tumor relapse. Proprotein convertases (PCs) are known to regulate the malignant phenotype of colon cancer cells by different mechanisms, but their effects on cancer stem cells (CSCs) have been less widely investigated. Here, we studied the PCs expression in colon CSCs, and the effect of their inhibition by using general PC inhibitors 1-PDX or decanoyl-RVKKR-chloromethylketone (CMK) on colon CSCs markers, growth, survival, and invasion in three-dimensional spheroid cultures. Moreover, Furin convertase was reported to be a pro-oncogenic driver in KRAS and BRAF driven colorectal cancer². We evaluated the specific repression of Furin in KRAS or BRAF mutant CRC cell lines and wild-type KRAS and BRAF on the expression of the stemness markers and global PCs activity.

P10 - Fascin-1 as a new potential target of aggressive hepatoblastoma

Lydia Dif

Hepatoblastoma (HB) is a liver tumor that arises in children. It's a sporadic malignancy that is often very aggressive. The current treatment consists of chemotherapy. However, chemotherapy in young patients has disastrous and long-term side effects such as ototoxicity, cardiomyopathy and infertility. Thus, alternative strategies are needed. One hint is to target the most common mutations in HB. It has been demonstrated that 90% of HB tumors are mutated for the Wnt pathway effector β -catenin. This mutation leads to an aberrant

constitutive activation of Wnt/ β -catenin signaling. However, β -catenin is an essential protein and is not a druggable target. Here, we investigate one of β -catenin targets, Fascin-1 that is found up-regulated in many tumors. Fascin1 affects actin organization into bundles and this leads to cell migration and invasion. Whereas Fascin-1 is absent from normal hepatocytes, we found its expression associated to the poor prognosis C2 subtype of HB. In both human and murine HB samples, Fascin-1 is associated to undifferentiated tumor cells. We further demonstrated that Fascin-1 expression modulates tumor hepatocyte differentiation status through gene expression. In this study, we investigate how Fascin-1 is able to regulate tumor cell plasticity and whether Fascin-1 is a druggable target in HB tumors. Our results show that the inhibition of Fascin-1 using siRNA strategy and a commercialized Fascin-1 inhibitor reduces cell migration, cell survival, and increases cell death in two HB cell lines (HepG2 and Huh6 cells). Moreover, we confirmed these results in HB patient-derived xenograft cells. To further understand the way Fascin-1 influences gene regulation, we analyzed the Hippo/YAP pathway, that plays a key role in HB development. We found that YAP activity is reduced after Fascin-1 depletion. Indeed, YAP is found translocated from the nucleus to the cytoplasm, upon Fascin-1 inhibition. In conclusion, our results show that Fascin could be an interesting druggable target in HB.

P11 - Snowflakes : a yeast model to study the maintenance of multicellularity

Tom Ducrocq

Multicellularity is present throughout the entire tree of life. Multicellularity has appeared and has been fixed in the tree of life at least 25 times in evolution history. The formation of multicellular entities can be explained by simple mechanisms, either by clonal development (cells are still cohesive after the end of mitosis) or by aggregation of cells from the same species. However, it is not fully understood how the first multicellular forms could be maintained or even selected. Yet, the recurrence of apparitions of multicellularity suggests that key selective drivers of simple multicellularity can exist [1]. Multiple phenomena have been assumed to drive the selection of simple forms of multicellularity, but very few experimental work has already tested those hypothesis. In this work, we use the Snowflake, a multicellular model of *Saccharomyces cerevisiae*, to test experimentally if environmental conditions and specific genetic background can be selective factors of simple forms of multicellularity. We specifically test if:

- Specific genes of the G1/S transition could be important for the maintenance of multicellularity.
- Respiration could be a selective factor of multicellularity.

P12 - How studying cell-to-cell gene expression variability could help deciphering endothelial-to-hematopoietic transition

Bérénice Dugué

Introduction: Differentiation is often conceptualized as a biological process resulting from the deterministic execution of programs encoded in the genome. According to this theory, all cells committed to a specific cellular fate follow the same instructions and therefore should exhibit minimal cell-to-cell gene expression variability. However, the importance of stochasticity in differentiation is increasingly recognized. It has recently been demonstrated that a peak of cell-to-cell gene expression variability occurs during avian erythropoiesis in vitro [1] and during adult human hematopoiesis in-vivo [2]. In this context, we aimed at demonstrating the importance of stochasticity in gene expression during another biological process: the endothelial-to-hematopoietic transition (EHT) (the process that allows generation of hematopoietic stem cells (HSC) during embryogenesis from specific endothelial cells of the dorsal aorta). **Methods:** Public Single-cell RNA-seq data from murine and human cells undergoing EHT were analyzed in order to assess cell-to-cell gene expression variability during the transition. We first reconstructed the differentiation trajectory of the cells and then measured cell-to-cell gene expression variability along the differentiation trajectory using Shannon Entropy as previously described [2]. **Results:** An increase in cell-to-cell gene expression variability is observed during EHT. Interestingly, this variability does not decrease after the transition, contrary to what was observed in adult hematopoiesis. Moreover, the genes whose cell-to-cell variation of expression increases the most over the course of EHT are enriched in transcription factors; some were already known to be involved in this process, but we also found ones that may be of interest for EHT. **Conclusion:** Our analysis confirms previous studies on the role of cell-to-cell gene expression variability during differentiation processes [1,2] and supports the stochastic view of differentiation. The cell-to-cell gene expression variability remains high at the end of EHT, suggesting that cells emerging from EHT are not as homogeneous as previously thought, which is in line with the study of Guibentif et al [3]. Some of the transcription factors with the biggest cell-to-cell variation of expression during EHT had not yet been described as having a role in this specific process ; therefore, it may be of interest to study the potential role of these transcription factors in experimental models of EHT. [1] : Richard, Angélique, et al. « Single-Cell-Based Analysis Highlights a Surge in Cell-to-Cell Molecular Variability Preceding Irreversible Commitment in a Differentiation Process ». PLOS Biology, vol. 14, no 12, déc 2016, p. e1002585. PLoS Journals, <https://doi.org/10.1371/journal.pbio.1002585>. [2] : Dussiau, Charles, et al. « Hematopoietic differentiation is characterized by a transient peak of entropy at a single-cell level ». BMC Biology, vol. 20, no 1, mars 2022, p. 60. BioMed Central, <https://doi.org/10.1186/s12915-022-01264-9>. [3] : Guibentif,

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P13 - Glycerol, a new key player in the trypanosome parasitic cycle

Mohammad El Kadri

Trypanosoma brucei is an extracellular parasite and the causative agent of African trypanosomiasis. The protozoan exhibits a complex life cycle taking place in an insect vector (procyclic forms, PCF) and a mammalian host (bloodstream forms, BSF) and requires morphologic and metabolic adaptations in order to survive in distinct environments. Until recently, BSF trypanosomes have been considered to propagate exclusively in the fluids of its mammalian hosts and mainly in the blood which is rich in glucose, widely considered by the scientific community as the only carbon source used by the parasite to feed its central carbon metabolism and its ATP production. However, we showed that BSF trypanosomes can efficiently grow in glucose-free conditions as long as glycerol is supplied (Pineda 2018; Bringaud 2021) and most parasites actually reside in the extravascular compartment of the skin and adipose tissue, where adipocytes excrete large amounts of glycerol from lipolysis and glycolysis (Capewell 2016; Trindade 2016). Therefore, we hypothesized that glycerol is a possible new player in the biology of African trypanosomes and the interactions between adipocytes and extravascular trypanosomes may confer a selective advantage to the parasites in the mammalian host. Our data shows that when glycerol is metabolized, it induces the differentiation of the proliferative slender BSF into growth-arrested or slow-growing forms that resemble stumpy BSF (forms preadapted to the insect environment), that can further differentiate efficiently to the PCF insect stage, suggesting a central role of glycerol in the transmission of the parasite from the mammalian host to the insect vector. It was confirmed by the failure of the glycerol kinase null mutant to differentiate to stumpy BSF with glycerol. These data support our hypothesis that the glycerol produced by adipocytes influences the metabolism of trypanosomes, which triggers its differentiation for transmission to the insect vector.

P14 - Targeting tryptophan metabolism as a new therapeutic approach in hepatoblastoma

Hala Fatrouni

Hepatoblastoma (HB) is the most frequent pediatric liver cancer, affecting children between 1 and 5 years old. Every year, 1 to 9 children per million develop this pathology which represent 1% of all malignant pediatric liver tumor with highly increased incidence during the last years. In term of therapy,

standard therapy is not efficient in 20% of cases and has many long lasting severe side effects underlining the need to find new and less toxic treatments. In many types of cancer, inhibiting the tryptophan (Trp) metabolism could be beneficial for the treatment as the kynurenine (Kyn) one of the metabolites of this pathway seem to have immunosuppressive and oncogenic functions. Surprisingly, this pathway is not active in HB, as key enzymes metabolizing Trp into Kyn such as tryptophan dioxygenase 2 (TDO2) are weakly expressed. In this project, we focused on TDO2 which we overexpressed in 2 different HB cell lines Huh6 and HepG2 and validated its metabolic activity. In this context, TDO2 overexpression led to a decrease in cell proliferation in both cell lines as assessed by 2D and spheroid cell culture 3D systems. We show that TDO2 was capable of inducing senescence as indicated by the increase of specific markers such as p16, p21 and β -galactosidase staining. Using electron microscopy, we noticed some morphological changes of mitochondria reflecting additional features typical of senescence. Our findings underscore a metabolic signature of Trp in hepatoblastoma which could be targeted as a new approach to treat this pediatric liver cancer.

P15 - Soluble guanylate cyclase stimulator pralicyguat promotes ischemic leg reperfusion in db/db mice

Ninon Foussard

Background: Lower-limb peripheral artery disease (PAD) is a prevalent complication of diabetes, requiring innovating therapies. Pralicyguat is an orally available stimulator of soluble guanylate cyclase (sGC) reported to have favorable effects on metabolic and hemodynamic endpoints in preclinical and clinical studies, suggesting the potential benefit in PAD. Objective: We evaluated the effect of pralicyguat on hindlimb ischemia recovery in a mouse model of diabetes. Method: Hindlimb ischemia was induced in db/db mice by ligation and excision of the left femoral artery. Pralicyguat 10mg/kg/day (N=10) or vehicle (N=10) were administered in the diet for 31 days starting 3 days before surgery. Foot perfusion was assessed with a Laser Doppler Imager and reported as a ratio in the ischemic versus non-ischemic limb. Ischemic leg function was assessed with a 4-point scale: 0, plantar/toe flexion in response to tail traction; 1, plantar but not toe flexion; 2, no flexion; 3, foot dragging. Results: Ischemic foot perfusion and function were better 28 days after surgery in pralicyguat than in vehicle treated mice (mean \pm SD: perfusion 1.05 ± 0.23 vs 0.38 ± 0.16 ; p

P16 - Repression of protein maturation inhibits pd-1 expression and enhances tumor clearance and tils: virtual ligand screen-ing and drug repurposing approach

Alexia François

Immune checkpoints, such as programmed death-1 (PD-1) are involved in the regulation of T cell effector function, are now exploited for the treatment of various solid and hematologic cancer. However, although therapies targeting PD-1 were clinically effective in various preclinical models and cancer patients, several patients with solid tumors are still refractory to these treatments. Indeed, solid tumors evade anti-cancer immune control by establishing immune privileged niches within the tumor microenvironment that reduce proliferation, viability, and/or activity of cytotoxic T lymphocytes (CTL). Interestingly, a wide range of proteins involved in the expression of PD-1 and CTL function require proteolytic activation by the proprotein convertases (known as PCs). Using general protein-based inhibitors of the PCs we previously reported the implication of the PCs in PD-1 expression and T cell exhaustion. In the current study we identified small molecule convertase inhibitors through virtual ligand screening and drug repurposing approach that inhibit the activity of the convertases. Using organoids culture, we found that some of these molecules were able to repress cancer cells viability, proliferation and invasion. These molecules were also able to mediate potent repression of PD-1 expression on T cells activated by CD3. In vivo, subcutaneous inoculation of mice with syngeneic cancer cells revealed their anti-tumoral efficacy that associated increased intratumoral T cell infiltration in the developed tumors. The treated mice showed improved overall survival while compared to controls. These and other findings highlight the potential use of PC inhibitors to increase the anti-tumoral immune response and could act as novel immunotherapeutic approach in cancer used alone or as adjunct therapy.

P17 - Integrative analysis of labeled targeted metabolomic and RNAseq transcriptomic data reveals metabolic plasticity in glioblastoma

Johanna Galvis

Glioblastoma (GB) is a malignant brain tumor with a low survival despite heavy treatment. In most of cancers, under low oxygen levels, aerobic glycolysis and fermentation predominate. To better characterize the role of lactate dehydrogenases (LDHA, LDHB), transcriptomics and stable isotope-resolved metabolomics (SIRM) were used. SIRM uses an isotope labeled substrate to track specific pathways. Most of the available bioinformatics pipelines are dedicated to conventional metabolomics (e.g. MetaboAnalyst), though commercial solutions for SIRM are available. We have developed DIMet (Differential Isotope targeted Metabolomics), a bioinformatics pipeline for differential analysis of

isotopic labeled data. The targeted metabolome ($^{13}\text{C}_6$ -glucose as substrate), to track glycolysis, TCA cycle and gluconeogenesis, and whole transcriptome, were obtained from P3 xenograft GBM cell cultures. P3 wild-type and double LDHA/B KO cells were exposed to different oxygen concentrations (0.1%, 21%). DIMet accepts isotopologues' contributions and full metabolites' abundances, and performs differential and time-series analyses. In the first one, Fold Change is computed for each metabolite, and statistical significance via t-test yields p-values which are adjusted for multiple comparisons (Benjamini-Hochberg). The threshold of significance for a Differentially Abundant Metabolite (DAM) is p_{adj}

P18 - TREM1+ CD163+ myeloid cells are potent immunosuppressive cells and associate with poor clinical factors in human liver cancer

Julie Giraud

Background. Hepatocellular carcinoma (HCC) is an inflammation-associated cancer and is among the deadliest cancers worldwide. Despite well-known risk factors, i.e. chronic viral infection with hepatitis B virus (HBV) primarily in Asia and HCV in western countries, excessive alcohol consumption and the metabolic syndrome-associated non-alcoholic steatohepatitis (NASH), HCC is diagnosed late in most patients (Llovet et al., 2021). The landscape of clinical trials for the treatment of advanced HCC has recently shifted to the field of immunotherapy and therapeutic options now includes the immune checkpoint inhibitors (ICI) nivolumab and pembrolizumab, and since 2020, the combination therapies (atezolizumab/bevacizumab and nivolumab/ipilimumab) (Finn et al., 2020). However, despite significant therapeutic advance with ICI, ~75% of patients do not respond to these immunotherapies for unclear reasons (Giraud et al., 2021). Recently, a meta-analysis of three randomized phase III clinical trials administering ICI to patients with advanced HCC showed a superior efficacy of immunotherapies in virally-infected patients compared to NASH-affected patients with HCC (Pfister et al., 2021). This suggests that the tumor microenvironment (TME) of HCC is an important determinant of therapeutic success and highlight the urgent need to further explore human liver-specific immunity towards the identification of theranostic immune biomarkers for patients' stratification and novel immunotherapies. Expansion of suppressive myeloid cells is a hallmark of chronic inflammation and cancer. Their heterogeneity in HCC is not fully resolved and might underlie immunotherapy resistance. Several studies have employed single cell analyses, including single cell RNA sequencing (scRNA-seq) and mass cytometry, to characterize the cellular landscapes of HCC. However, the bulk of these studies included all liver cells, limiting the granularity of the analysis. **Objective.** In this study, we setup to discriminate and localize human liver-specific innate immunity cells to improve the stratification and

the treatment of patients with HCC. **Methods.** We implemented scRNA-seq on purified CD45+panTCR $\alpha\beta$ -CD19- cells freshly isolated from tumoral and juxta-tumoral tissues from 10 patients with HCC of different etiologies, and performed spatial transcriptomics (stRNA-seq) (10x Genomics) to map their localization. We validated our results by multiplex immunofluorescence, by functional analyses performed on ex-vivo FACS-sorted cells co-cultures, on a mouse model of HCC, and by computational analyses of published HCC data sets. **Results.** Here, we report a high-resolution atlas of innate immunity cells (around 100,000 transcriptomes) in HCC and unravel a strong myeloid bias in NK cell differentiation and a remarkable myeloid cell heterogeneity. In particular, we identify three phenotypically distinct THBS1+ monocytes, including a distinct population expressing a variety of myeloid lineage-affiliated genes and selectively marked by elevated expression of triggering receptor expressed by myeloid cells-1 (TREM1) in conjunction with CD163. We show that TREM1+CD163+ myeloid cells is a potent immunosuppressive subset ex vivo and expand in models of liver inflammation and fibrosis in vivo. The proportion of specific gene signature defining TREM1+CD163+ myeloid cells is higher in HCC compared to non-tumoral tissues and correlate with poor patient clinical factors in HCC and response to immune checkpoint blockade. We further show that TREM1+CD163+ myeloid cells are high producers of TGF β and spatially localize at liver fibrotic lesions in close association with cancer associated fibroblasts. **Conclusion.** Collectively, our data support for a myeloid subset-targeted immunotherapies to treat HCC.

P19 - ARSENAL: Antimicrobial ReSistance prEdictionN by a mAchine Learning method

Ulysse Guyet

Antimicrobial resistance (AMR) has become a major public health concern due to the rapid emergence of multidrug-resistant bacteria, causing serious problems for the prevention and treatment of persistent infections. Development of algorithms for phenotypic variation prediction, such as AMR, could be of major clinical importance, more reliable and efficient compared to traditional phenotyping, and could contribute to the discovery of previously unknown AMR pathways. Significant increase of the available sequencing and associated phenotypic data in recent years creates the basis for the development of such methods. Here, we developed a machine learning method -ARSENAL- for predicting the minimum inhibitory concentration (MIC) of several antibiotics based on genomic data. ARSENAL relies on one hand on the sequence (k-mers), and on the other hand on the genome structure (gene composition) and the gene orthology links between the strains of the same species. Functional interpretation of the most predictive features confirmed the biological relevance of the ARSENAL model.

P20 - TbPat is a patatin-like phospholipase localized in the lipid droplets of Trypanosoma brucei

Perrine Hervé

Patatin-like phospholipases (PLPs) are a group of phospholipases A2 (PLA2), which hydrolyze phospholipids at the sn2 position into free fatty acids (FAs) and lysophospholipids. In pathogens such as bacteria (*Pseudomonas aeruginosa*) or protozoan parasites (*Toxoplasma*, *Plasmodium*), PLPs have been shown to be involved in their pathogenicity and virulence. Most PLPs possess conserved domains : a glycine-rich oxyanion hole, a catalytic serine surrounded by glycines (GxSxG) and a conserved proline with an associated motif. By mining the genome database of *Trypanosoma brucei*, a protozoan parasite responsible for sleeping sickness, we found a single gene that possesses all these PLPs domains. We therefore aimed to further analyze this new gene, TbPat. Using CRISPR-Cas9 and plasmid constructions, we found that TbPat is localized in lipid droplets (LDs), a compartment involved in many cellular processes such as FA metabolism. Expression of TbPat was increased when adding FAs into the culture medium, coinciding with an increase in the number of LDs in parasites. TbPat is the first PLA2 candidate in *T. brucei* and a potential marker for LDs, a compartment poorly characterized in trypanosomes.

P21 - Single cell RNAseq analysis of human CD34+ cells during their ex vivo expansion: Towards a better understanding and control of self-renewal process

Mathilde Huart

Haematopoietic stem cells (HSCs) that are the origin of all mature blood cells can divide in 2 conceptually different modes: symmetric or asymmetric way. The study and understanding of these mechanisms is essential for basic knowledge, but also in the context of ex vivo graft expansion that leads to stem cells exhaustion due to uncontrolled stem cells division and differentiation. Recently, we showed that the addition of a c-Jun N-Terminal kinase inhibitor (JNK-IN-8) and the anti-oxidant molecule alpha-lipoic acid (ALA) allows to increase up to 5-fold the steady state peripheral blood primitive CD34+ cells pool during expansion procedure while preserving their engraftment capacity. These results suggest that the combination of JNK-IN-8 and ALA allows for the amplification of stem cell quantity during the expansion procedure, potentially by promoting symmetric division based self-renewal. In order to investigate the molecular pathways involved in these processes, we carried out an in-depth study of the transcriptome using a single cell approach via the Chromium technology (10x Genomics). For this experiment we performed several libraries; 1 library at D0 in order to have a global image of the freshly isolated cells. 4 libraries: CTRL, JNK-IN-8 and/or ALA after 6 hours of culture in expansion medium in order to determine the immediate action

and the signalling pathways preferentially affected by the molecules alone or in combination. And 2 libraries CTRL and JNK-IN-8 + ALA (combination) at day 12 in order to determine the final molecular state of the cells before transplantation. The first results at 6 hours show an immediate action of JNK-IN-8 on the cytoskeleton regulation and dynamic. Whereas ALA seems to promote the expression of genes involved in WNT signalling pathways known for its action on stem cells maintenance. The results at day 12 tend to confirm these observations and allowed us to confirm the effects of the combination on the non-canonical WNT/PCP pathway with a significant decrease in the genes involved in this pathway. We can then hypothesize that JNK-IN-8 and ALA act in a dissociated but complementary manner. Indeed, JNK-IN-8 could modulate the regulation of the cytoskeleton, allowing for a correct cell morphology and division dynamics favouring self-renewal by symmetric division. As the fate of daughter cells is mainly dictated by the molecular inheritance obtained during cytokinesis, ALA, when present, would increase the expression of genes involved in the maintenance of stem cell properties, notably the canonical WNT β -catenin pathway. Combined together, it appears that these molecules allow the enrichment of functional stem cell culture.

*P22 - The cytolethal distending toxin from *Helicobacter hepaticus* modulates the Hippo signaling pathway*

Ruxue Jia

We are frequently exposed to infection with genotoxin-producing bacteria, such as cytolethal distending toxin (CDT), a prevalent heterotrimeric toxin among Gram-negative bacteria. CDT causes severe DNA damage in host cells, a well-known risk factor of cancer development and progression. Numerous data point for an etiological role of CDT in carcinogenesis. Indeed, CDT from *Helicobacter hepaticus*, via its active CdtB subunit, was shown to be involved in the development of murine hepatocarcinoma. We previously showed that CDT modulates cell differentiation and elicits epithelial to mesenchymal transition (EMT), a process by which cells lose their epithelial characteristics in favor of mesenchymal ones, conducive to cell motility. The evolutionarily conserved Hippo signaling pathway is involved in EMT and metastasis. In the present study, we thus investigated the effect of CDT on the Hippo signaling pathway following *H. hepaticus* CdtB subunit expression. These investigations were performed in vitro on human epithelial cell lines upon ectopic expression of *H. hepaticus* CdtB and its corresponding mutated CdtB lacking catalytic activity (CdtBMut), to attribute the observed effects specifically to the CdtB. Some results were also confirmed in vivo using xenograft mouse models following *H. hepaticus* CdtB or CdtBMut expression. In vitro 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 (Yes-associated protein 1) and TAZ (WW Domain Containing Transcription Regulator 1). Increased transcriptional enhanced associated domain (TEAD) activity was shown upon CdtB expression using the TEAD reporter assay. Verteporfin, a compound preventing YAP1/TAZ-TEAD interaction, reduced CdtB-increased nuclear remodeling and CdtB-increased TEAD transcription activity, confirming the involvement of CdtB in the regulation of Hippo signaling pathway. An increase of LATS2, YAP1 and phosphorylated YAP1 was observed in vivo in the CdtB-expressing tumor using the xenograft mouse models. Taken together, these data show that CDT/CdtB activates the Hippo signaling pathway supporting the idea that infection with CDT-producing bacteria can promote cancer development.

P23 - Serglycin at the Glia Limitans, a key player of neuro-inflammation pathophysiology

Margaux Laisné

During neuropathology, notably multiple sclerosis (MS), Blood Brain Barrier breakdown leads to parenchymal inflammatory infiltration. Recently, we highlighted the capacity of astrocyte to communicate with other neurovascular unit cells, producing pro-inflammatory and pro-permeability factors. We performed an RNA sequencing on quiescent versus reactive human astrocytes (hRA) and identify serglycin (SRGN) as highly expressed by hRA. We confirmed these results in vivo in human MS lesions and in Experimental Auto-immune Encephalomyelitis mice model. Our goal is to unravel the contribution of SRGN at the Glia Limitans in neuro-inflammatory condition. Our hypothesis is that SRGN through its interaction with the CD44 cell-surface receptor modulates astrogliosis and immune cell infiltration during MS neuro-inflammation. We treated hRA in vitro with SRGN siRNA (siSRGN) to investigate the impact of SRGN at a cellular and molecular level. We observed a decreased expression of CD44, hRA cytoskeleton marker (vimentin), pro-inflammatory factor (IL-6) in siSRGN treated hRA. Moreover, using immunofluorescence, we observed a modification in CD44 cellular localization and a reorganization of the cytoskeleton in hRA treated with siSRGN compared to control. Finally, we observe that SRGN promote hRA migration. Collectively, these data suggest that SRGN upregulation in reactive astrocytes promotes cellular and molecular changes at the glia limitans under neuroinflammatory condition.

P24 - The trypanosome flagellar pocket cytoskeleton: building an in silico, in vitro, and in vivo interactome to understand its biogenesis and function

Chloé Lambert

The trypanosome flagellar pocket cytoskeleton: building an in silico, in vitro, and in vivo interactome to understand its biogenesis and function. Elina Casas¹, Chloé Lambert¹, Nicolas Landrein¹, Gang Dong², Denis Dacheux^{1,3}, Derrick R. Robinson¹, Mélanie BONHIVERS¹ 1 MFP, CNRS UMR5234, University Bordeaux, 2 Max Perutz Labs, Vienna BioCenter 3 MFP, Bordeaux INP Trypanosoma brucei belongs to a group of single-celled, flagellated parasites that are responsible for human and animal African trypanosomiasis (sleeping sickness). These cells have a flagellum that exits the cell body via the flagellar pocket (FP). The FP is the only site of endo/exocytosis, and as such is essential and a potential target. The flagellar pocket collar (FPC) is a cytoskeletal structure that keeps the FP closed and functional. A better understanding of the composition, structure and function of the FPC is required to understand the biogenesis and function of the FP. We have previously characterized the first FPC protein - BILBO1 as an essential protein for FPC and FP biogenesis (1). The recent developments in expansion microscopy have facilitated the refinement of protein localisation in the parasite and to identify structures that were not previously observed. We show here, using Ultrastructure expansion microscopy and BILBO1 labelling, that FPC biogenesis occurs de novo, which allows us to better understand the formation of the FP during the cell cycle. BILBO1 forms a skeleton onto which several partners anchor including BILBO2 (2-4). The N-terminal domains (NTDs) of BILBO2 and BILBO1 are similar in structure and function (3). We have recently identified 3 other proteins (BILBO3, 4, 5) with a similar NTD. These proteins form a previously uncharacterized trypanosome-specific protein family. Immunolocalization combined with proximity-dependent biotin identification of BILBO3 partners suggests that the NTDs provide an anchor domain to the proteins trafficking from the basal bodies to the FPC and help the understanding of the interactome of cytoskeletal structures associated with the FP. 1. Florimond, C. et al. PLoS Pathog. 11, (2015) 2. Albisetti, A. et al. PLOS Pathog. 13, e1006710 (2017) 3. Isch, C. et al. PLOS Pathog. 17, e1009329 (2021) 4. Perdomo, D. et al. Parasite 29, 14 (2022)

P25 - Refining the Role of Pyruvate Dehydrogenase Kinases in Glioblastoma Development

Claire Larrieu

Glioblastoma (GB) are the most frequent brain cancers. Aggressive growth and limited treatment options induce a median survival of 12-15 months. In addition to highly proliferative and invasive properties, GB cells show cancer-associated metabolic characteristics such as increased aerobic glycolysis.

Pyruvate dehydrogenase (PDH) is a key enzyme complex at the crossroads between lactic fermentation and oxidative pathways, finely regulated by PDH kinases (PDHKs). PDHKs are often overexpressed in cancer cells to facilitate high glycolytic flux. We hypothesized that targeting PDHKs, by disturbing cancer metabolic homeostasis, would alter GB progression and render cells vulnerable to additional cancer treatment. Using patient databases, distinct expression patterns of PDHK1 and PDHK2 in GB tissues were obvious. To disturb protumoral glycolysis, we modulated PDH activity through the genetic or pharmacological inhibition of PDHK in patient-derived stem-like spheroids. Striking effects of PDHKs inhibition using dichloroacetate were observed in vitro on cell morphology and metabolism, resulting in increased intracellular ROS levels and decreased proliferation and invasion. In vivo findings confirmed a reduction in tumor size and better survival of mice implanted with PDHK1 and PDHK2 knockout cells. Adding a radiotherapeutic protocol further resulted in a reduction in tumor size and improved mouse survival in our model.

P26 - Elucidating the role of N-Acetyl-Aspartate in normal hematopoiesis

Claire Lauvinerie

Mature blood cells have a limited lifespan and must be continually renewed. At the top of this hierarchical process are hematopoietic stem cells (HSC). It is well established that adult bone marrow (ABM) HSC are mainly quiescent. Conversely, HSC in the fetal liver (FL) proliferate extensively, calling for distinct bioenergetic requirements. The link between metabolism and functional capacities of HSC have mostly been studied in adult mouse models. Hence, knowledge on proliferating HSC remains elusive. To fill this gap, we conducted a metabolomic comparative analysis of quiescent ABM-HSC and proliferative FL-HSC. Our interest rapidly focused on a metabolite with a 10-fold higher abundance in FL-HSC than in ABM-HSC: the N-Acetyl-Aspartate (NAA). Although NAA is the second most prevalent metabolite in the brain, its function is still unclear. To investigate the role of NAA in adult and fetal hematopoiesis, we have used a transgenic mouse model knockout for *Nat8l*, the gene coding for N-Acetyl-Aspartate Transferase, the enzyme catalyzing the production of NAA in cells; in this mouse model NAA is not detectable in any tissue. Using flow cytometry, we have characterized the bone marrow of *Nat8l*^{-/-} and *Nat8l*^{+/+} adult mice, as well as the FL of E14.5 embryos. We have also conducted competitive hematopoietic reconstitution assays with both genotype to assess the role of NAA in HSC function. We have shown that NAA is dispensable for both adult and fetal normal hematopoiesis, but might be implicated in stress hematopoiesis.

P27 - Involvement of the transcription factor NRF1 in Alpha1-Antitrypsin Deficiency mediated liver damage

Alexandra Lehmann

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 (E342K) that leads to the retention and the accumulation of Z aggregates into the ER causing cirrhosis and liver cancers. 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. 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 decreased, is up-regulated only in cells expressing the Z variant, suggesting that the Z variant mediates proteasome impairment compensated by NRF1 activation. In order to evaluate the impact of a loss of NRF1 into AATD mediated liver damage, we are generating, using Crispr-Cas9 technology, NRF1 KO cell lines. Next, we will characterize the modulation of NRF1-proteasome axis in AATD liver disease and explore its modulation as a therapeutic strategy.

P28 - Identification of autophagy actors involved in the Alpha 1-antitrypsin deficiency

Céline Leon

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 (E342K) that leads to the retention and the accumulation of Z aggregates into the ER triggering intracellular injury cascade, cell death and chronic liver damage (cirrhosis and liver cancers). While liver transplantation is the only curative treatment available at this time, 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. Our hypothesis is that an impairment in this pathway could lead to Z proteotoxicity and then to liver damages. However, so far the ERphagy pathway involved in Z-AAT degradation is poorly characterized, little is known about all the actors. Therefore, 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 set up a high throughput assay 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 with flow cytometry. Next, based on this system, 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.

P29 - Deep Learning-based Pipeline for the Quantification of Synthetic Fluorescence Images of Spheroids

Christer Lohk

In recent years, cell spheroid cultures have found wide use in biomedical research for the quantification of the drug effect, for example using simple metrics such as cell count, volume or frequency of cell events. Here, we propose a pipeline to extract advanced image features from 3D images of nuclei. We aim to maximize the performance of StarDist, a supervised deep learning algorithm for nucleus segmentation by generating a large representative training image set with their corresponding annotations. In addition, we use our pipeline for nucleus feature extraction to estimate the performance of a second deep learning network that generates synthetic images of spheroids. The proposed generative network would enrich the segmentation model with a new dataset including synthetic images. By using a more accurate segmentation model, we expect to increase the overall performance of the feature extraction pipeline.

P30 - Development of an «Intestine-On-chip» to study infections by opportunistic pathogenic yeast Candida

Fernanda Lopez Garcia

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 under flow 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 ($\sim\mu\text{m}$) 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. References Cunningham, M.L. (2002). A Mouse Is Not a Rat Is Not a Human: Species Differences Exist. *Toxicol. Sci.* 70, 157–158. Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., and Ingber, D.E. (2010). Reconstituting organ-level lung functions on a chip. *Science* 328, 1662–1668

P31 - PCSK9 expression on LSEC during Liver Metastasis

Ander Martin San Sebastian

The protein-convertase PCSK9 has been extensively studied in hypercholesterolemia but has recently been shown to be involved in cancer. This protein of the convertase family is overexpressed in several types of cancers, especially during the process of metastasis. One of the most frequent is colon liver metastasis, a process in which colon tumor cells migrate to the liver to create a secondary tumor. In this process of tumor dissemination, the tumor microenvironment plays a key role. Our research focus on the function of PCSK9 in hepatic sinusoidal endothelial cells (LSEC). We have shown in an in vitro study that PCSK9 protein is expressed in LSECs under basal conditions. Additionally, we have shown that activation of LSECs with cancer stem cell (CSC)-conditioned media significantly increases PCSK9 expression at mRNA level. Furthermore, PCSK9 immunofluorescence staining has shown nuclear localization in LSECs in cell culture which was confirmed by western blotting of the cell nuclear fraction. PCSK9 has also shows nuclear staining in healthy and tumor tissues, which opens the door to a large number of new functionalities of this protein. Finally, the proliferative capacity of LSECs is reduced when PCSK9 is chemically inhibited, which could lead to decreased formation of new blood vessels to irrigate the tumor. In summary, PCSK9 appears to play a protumoral role in LSECs during liver metastasis and might have an unknown nuclear function.

P32 - Actin Cytoskeleton In Quiescent Yeast Cells

David Mauboules

Quiescence, defined as a transitory proliferation arrest, is the most widely spread cellular state on earth. Quiescence is central for major biological processes such as development and tissues homeostasis. Its deregulation is at the origin of many human diseases such as cancer or age related degenerative diseases. Yet, quiescence is still poorly understood. Recent works on the yeast *Saccharomyces cerevisiae* have shown that quiescence establishment is associated with the reorganization of many organelles and cellular machineries. One of these remodeling concerns the actin cytoskeleton. In proliferating yeast, the actin cytoskeleton is organized in highly dynamic structures: cables required for polarized growth, patches responsible for endocytosis and the cytokinesis ring. When yeast enter quiescence, these structures vanish and an original structure composed of stable actin filaments is formed. During my PhD, I will combine in vivo imaging and yeast genetics to understand the molecular mechanisms underlying the formation and the stabilization of this quiescence specific actin structure.

P33 - A simple core model of metabolism around mitochondria

Jean-Pierre Mazat*

We developed a reduced metabolic model of central carbon metabolism and nitrogen, C2M2N with 77 reactions, 53 internal metabolites and 3 compartments, taking into account the true stoichiometry of the reactions, including the stoichiometric role of the cofactors and the role of proton gradient in oxidative phosphorylation (OXPHOS). To illustrate the interest of such a reduced model of metabolism in mammalian cell, we use Flux Balance Analysis (FBA), to systematically study all the possible fates of glutamine in central carbon metabolism. We demonstrate that glutamine can supply other sources of carbon for cell energy production and as carbon source to synthesize the different essential metabolites thus sustaining cell proliferation. We also show the role of reductive glutamine pathway to the rescue of oxphos defect and hypoxia. More generally, we explore the metabolism rewiring in mitochondrial defects and the ways to bypass them. With the addition of a core submodel of folate pathways (C2M2NF) and the concept of the “average cancer cell”, we explore the salient features of the 1C metabolism in proliferating cells and its relationships with the rest of metabolism. All these studies show that: - NADH regeneration is the big problem of proliferating cell - The NAD(P)/NAD(P)H ratios are different in cytosol and mitochondria thus inducing differences between the substrate/product ratios involved in the corresponding dehydrogenases. For this reason, the NAD(P)/NAD(P)H ratios operate as the main switches between the different metabolic pathways in the different compartments. - The concept of “average

*out of competition

cancer cell”, even if it does not exist as such, allows us to understand the main features of the cancer cell metabolism.

P34 - Oculocutaneous albinism: characterisation of pathophysiological mechanisms and validation of new pathogenic variants

Elina Mercier

Oculocutaneous albinism (OCA) is a rare clinically and genetically heterogeneous disease, combining hypopigmentation and severe visual impairment. This project focuses on 5 types of OCA and corresponding genes, involved in pigment synthesis: OCA1 (TYR), 2 (OCA2), 3 (TYRP1), 4 (SLC45A2) as well as OCA8 (TYRP2/DCT) recently identified in the laboratory. The links between pigmentation alterations and developmental abnormalities of the retina leading to visual deficits are still not understood. L-Dopa, an early intermediate in pigment production, named melanogenesis, is synthesized by hydroxylation of tyrosine by tyrosinase (TYR). It is produced in the retinal pigment epithelium (RPE) during embryogenesis and thought to be instrumental for retinogenesis, independently of pigment production. Functional explorations of loss of function of TYRP1 and SLC45A2 in a cellular model revealed that L-Dopa is undetected. Moreover, L-Dopa is significantly reduced in postnatal Dct^{-/-} mouse optic cups and undetected in the developing RPE. This suggests that TYRP1, SLC45A2 and Dct may positively regulate tyrosinase activity. Therefore, the first objective of this project is to elucidate these interactions and to establish the molecular links between genetic abnormalities, functional pathways and retinal alterations. The search for these links will be based on the functional exploration of different model systems: melanocytes in culture, transgenic mouse lines, RPE cells derived from patient iPSCs, Xenopus embryos. The second objective is to improve diagnosis of OCA patients by characterizing candidate variants. Indeed, 30% patients remain unsolved or with incomplete diagnosis after analysis of the known OCA gene coding sequences. For these patients, rare variants of unknown significance will be functionally tested in cultured melanocytes. This strategy should increase our knowledge on genotype to phenotype relationship as well as our understanding of the pathology.

P35 - Impact of type-I interferons on melanocytes phenotype and function in vitiligo

Ribal Merhi

Vitiligo is an auto-immune skin depigmenting disease characterized by the loss of epidermal melanocytes, the skin pigmented 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- α and IFN- β) on melanocyte phenotype and function during vitiligo pathogenesis. As previously described, vitiligo skin is infiltrated with IFN- α producing plasmacytoid dendritic cells. Our results show that keratinocytes stimulated with Toll-Like Receptor 3 agonists produce IFN- β . In addition, the expression of interferon-alpha receptor (IFNAR) by melanocytes is increased in the 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. In vitro experiments revealed that melanocytes express a functional IFNAR. IFN- α and IFN- β treated melanocytes show cell cycle arrest, increased expression of Senescence Associated Beta-galactosidase, phosphorylated-p53, p16INK4a, and p21CIP/WAF1. Furthermore, levels of IL-6 and IGFBP-3, two major components of the senescence-associated secretory phenotype, were increased in the presence of type I IFNs. Additionally, our RT-qPCR results suggest that neither IFN- α nor IFN- β 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.

P36 - Improving Chimeric Antigen Receptor (CAR) T lymphocyte efficacy in breast cancers: reprogramming of the tumor microenvironment with hormonotherapy
Alexandra Moisand

Following its recent success in the treatment of hematological malignancies, Chimeric Antigen Receptor (CAR) T lymphocyte-based immunotherapy has been thrust in the limelight. In contrast with these observations, the clinical efficacy of CAR T cells in the context of solid cancers has yet to be proven and associated hurdles need to be overcome. Of note, the immunosuppressive microenvironment characteristic of such cancers is known to impede the activity of CAR T cells, thereby highlighting the critical need for its reprogramming. Here, using a murine model of breast cancer, we have evaluated the potential of hormonotherapy in reprogramming the immunosuppressive microenvironment and its impact on the efficacy of CAR T lymphocytes. Second-generation IL13R α 2 murine CAR T cells were designed by our host laboratory (City of Hope, CA, USA) and a platform for their generation was developed. Efficacy of IL13BB ζ CAR T against breast cancer cells expressing the cognate IL13R α 2 antigen was evaluated prior to their implementation in vivo, where the immunotherapy was administered in combination with

the anti-estrogen Fulvestrant. Primary tumor and metastatic disease were assessed throughout, and immunosuppressive populations of Myeloid-Derived Suppressor Cells (MDSC) and regulatory T lymphocytes (Tregs) and effector CD4 and CD8 T lymphocytes within primary tumors and metastatic sites were monitored without revealing significant differences upon various treatments. Although the presented results are preliminary in nature, they nevertheless warrant further investigations.

P37 - Delta-like 4 at the glia limitans, a key player of neuro-inflammation pathophysiology

Pierre Mora

Introduction: During neuro-inflammation, astrocytes become reactive. They undergo morphological and molecular changes named “astrogliosis” and drive the conversion from acute inflammatory injury to a chronic neurodegenerative state. To characterize astrocyte signature during astrogliosis, a RNA sequencing on quiescent versus reactive astrocytes was performed. We identified delta-like 4 (Dll4) as highly expressed by reactive astrocytes. **Aims:** Our goal is to unravel the contribution of Dll4-Notch1 signaling to astrogliosis and ensuing neurovascular unit destabilization. **Methods:** We induced chronic and acute neuro-inflammation in conditional astrocytic Dll4 knock-out (Dll4ACKO) mice and control littermates. Lesion size, inflammation, demyelination and clinical disability were measured. In parallel, human reactive astrocytes were treated with a Dll4 siRNA to study mechanism. **Results:** In vivo, Dll4ACKO mice exhibited a milder pathology and astrogliosis than controls. It was correlated to the decreased expression of IL-6 and pro-permeability factors. This was confirmed in vitro on reactive astrocytes knockdown for Dll4. To go deeper into the mechanism, we demonstrated that the Dll4-Notch1 juxtacrine signaling in reactive astrocytes directly controls IL-6 transcriptional level and that blocking IL-6 receptor decreases astrogliosis and pro-permeability factor expression. **Discussion:** Collectively, these data suggest that the Dll4-Notch1 signaling drives astrogliosis during neuro-inflammation via IL-6 up regulation promoting neurovascular unit disruption and pathology severity.

P38 - Characterization of Nrf2 Role in the Development and Therapeutic Resistance of Human Glioblastoma

Maya Moubarak

The nuclear factor erythroid 2-related factor 2 (Nrf2), a CNC-bZip transcription factor encoded by the NFE2L2 gene, acts as a master regulator of human antioxidant defenses. Although Nrf2 is expressed at low levels in all cell types, it regulates more than 200 genes to control cellular function, exhibit anti-inflammatory actions, and maintain cellular redox homeostasis. Unfortunately,

constitutive Nrf2 activation promotes cancer growth, treatment resistance, and poor prognosis in a variety of cancer types. Glioblastoma (GB), WHO grade IV glioma, is the most prevalent primary malignant brain tumor characterized by a short survival rate after diagnosis. Despite some therapeutic options, recurrent GBM management remains a challenge with limited treatment options. Glioma stem cells (GSCs) constitute a small fraction of the tumor bulk yet they possess neural stem cell properties and high self-renewal capacity allowing for tumor growth and therapeutic resistance. Overexpressed Nrf2 in human GBM tissues associates with shorter survival, diminished differentiation progress, and self-renewal capacity of GSCs. Hence, the role of Nrf2 in GSC stemness and therapy resistance is yet to be mechanistically determined. Our study first aims to investigate Nrf2 as a potential therapeutic candidate to target the self-renewal features of GSC using patient-derived GB cells in 3D culture models. Second, we aim to investigate Nrf2's potential for targeting GSC stemness and therapy resistance by assessing stemness markers and TMZ sensitivity in Nrf2 wildtype and CRISPR/Cas9 Nrf2^{-/-} of GB patient-derived cells in 3D models. Finally, we aim to investigate our approach in vivo using orthotopic injections of patient-derived cell xenografts in immunodeficient mice. Therefore, our work will provide a substantial benefit by identifying the role of Nrf2 in GB progression and therapeutic resistance which may improve the outcomes of GB cancer therapy.

P39 - A novel transgenic model to study the stem cells that persist upon tyrosine kinase inhibitor treatment of chronic myeloid leukemia

Amal Nazaraliyev

Chronic myeloid leukemia (CML) is a myeloproliferative disease driven by BCR-ABL1, a fusion oncogene that encodes a constitutively active tyrosine kinase. CML is thought to arise when a hematopoietic stem cell (HSC) acquires BCR-ABL1, leading to transformation and the development of leukemia. The discovery of tyrosine kinase inhibitors (TKIs) that block BCR-ABL1 activity has improved CML outcomes. However, lifelong treatment is required as TKI cessation leads to relapse in >50% of patients. Relapse has been attributed to a population of leukemic stem cells (LSCs) that persists despite the presence of TKIs and give rise to disease when left unchecked. However, the study of such LSCs has been hindered by the lack of models that recapitulate the human pathology, notably the chronic phase of CML. We believe that the differences from the human pathology are primarily due to non-specific expression of BCR-ABL1 outside of the pool of HSCs. To this end, we recently established a novel CML model that enables the inducible expression of a BCR-ABL1 transgene specifically within a fraction of HSCs. Importantly, this transgenic reporter system permits the identification and distinction between normal HSCs and cells that express BCR-ABL1 through differential expression of fluorescent

reporters. We investigated long-term impact of BCR-ABL1 expression through continuous analysis of BCR-ABL1-expressing animals. We found that the absolute numbers of platelets and white blood cells gradually increased in mice expressing BCR-ABL1 compared to controls. Flow cytometric analyses of hematopoietic stem and progenitor cells (HSPCs) as well as lineage-restricted and mature cell populations showed a rapid expansion of leukemic cells in HSPCs followed by an increase in leukemic myeloid cells in the peripheral blood. The increase in leukemic cells detected in each population occurred at the expense of its normal hematopoietic counterpart. While this expansion of leukemic cells occurred within the first 100 days following induction of BCR-ABL1 expression, the survival of these mice was prolonged for 3-4 times (average ~275 days) compared to that of other in vivo CML models (60-70 days). Pilot studies of TKI treatment of our CML animals showed a reversal of some effects and restoration of the stem cell compartment suggesting that our model can be used to isolate and study LSCs.

P40 - Targeting PI3K/AKT/mTORC1 signalling in gastric cancer stem cells

Tra Ly Nguyen

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. In this project, we have identified the upregulation of PI3K/AKT/mTORC1 pathway in our omics data, then we would like to study the role of this signalling on 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 tumor growth and dissemination on different sub-populations of GCSCs.

P41 - Microvascular impairment in heart failure with preserved ejection fraction

Nabil Nicolas

Heart Failure with preserved Ejection Fraction (HFpEF) is a cardiovascular disease characterized by diastolic dysfunction. Main risk factors include advanced age, sex (women being more susceptible), and comorbidities like obesity, type 2 diabetes, and renal dysfunction. The etiology of the disease is poorly known. Our pathophysiological hypothesis is that metabolic disorders and renal dysfunction induced by the risk factors generate a systemic pro-inflammatory state and hemodynamical changes that, accumulating with age,

are responsible for endothelial dysfunction and pathological reorganization of the microvascular morphology, leading to diastolic dysfunction. The objective of the study was, using an HFpEF mouse model, to (1) identify the metabolic disorders and the renal dysfunction, (2) evaluate the pro-inflammatory state, (3) quantify the cardiac microvascular morphology, and (4) characterize the cardiac diastolic function. Experiments were done on 14-week-old female C57BL/Ks mice, predisposed to renal dysfunction, deficient for leptin receptors, and hence developing obesity and type 2 diabetes. 8-week-old male C57BL/6J mice, lacking HFpEF risk factors, were used as healthy control. Glycemia, triglyceridemia, cholesterolemia, and % pro-inflammatory cells were analyzed on blood samples. 3D imaging of glomeruli was done by light-sheet microscopy (LSM) on optically cleared kidneys. 3D imaging of the coronary capillary network was done by LSM on optically cleared hearts. Diastolic pressure, developed pressure, and relaxation rate was measured on Langendorff's isolated perfused hearts. Compared to controls, HFpEF mice showed increased plasmatic glucose, triglyceride, and cholesterol concentration, and increased monocyte and granulocyte %. Their kidneys had decreased glomerular volume, and compactness. The coronary capillary network was altered in the left and right ventricles. Isolated hearts showed left ventricle diastolic dysfunction. Our study suggests that risk factors induce diastolic dysfunction related to coronary microvascularisation morphological alteration via a systemic pro-inflammatory state. Further studies are needed to investigate hemodynamic alteration and endothelial dysfunction.

P42 - Rho GTPase nanoclustering ensures the appropriate ordering of cell cycle events

Landry Peyran

Healthy cell proliferation requires the correct ordering of cell cycle events and the monitoring of these events via checkpoints that delay cell cycle progression when problems are encountered. We have discovered that the establishment of a polarity axis is a key event that controls the correct ordering of the cell cycle. As in all eukaryotes, polarity axis establishment in budding yeast requires the activation of the Rho GTPase Cdc42, which governs the axis along which cells grow and divide. Defects in polarity trigger a Swe1 (Wee1)-dependent checkpoint that delays mitotic entry via Cdk1 inhibitory phosphorylation. Using specific mutations that perturb cell polarity, we observe catastrophic cell cycle defects including cell cycle misordering. In contrast to WT cells in which successive waves of Cdk1 activity associated with different cyclins impart temporal order on cell cycle events, the polarity mutant is characterized by considerable G1, S and M phase cyclin overlap. The biological consequences of this misregulation include the misordering of G1, S and M phase events and the accumulation of multinucleate cells. These dramatic cell cycle defects accumulate despite

robust Swe1 (Wee1)-dependent inhibitory phosphorylation of Cdk1 and are associated with a novel signal emanating from G1 cyclins. 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.

P43 - Prognostic role of proprotein convertases in pancreatic ductal adenocarcinoma (PDAC)

The pancreatic ductal adenocarcinoma (PDAC) is a major health issue with a 5-year relative survival rate of only 6%. This aggressiveness is mainly due to a late diagnosis and a lack of curative option with a resistance to most conventional treatment (chemotherapy and radiotherapy). Interestingly, the activation of the majority of the signaling pathways involved in the initiation and progression of pancreatic cancer is mediated by various protein precursors that require proteolytic activation by a family of nine enzymes called proprotein convertases (PCs). Consequently, deregulation in the expression and activity of proprotein convertases (PCs) is associated with pathological conditions and they are known to behave as oncogenes in various types of cancer. Indeed, we have found that four members of PC family are predominantly expressed in PDAC tissues while compared to noncancerous tissues. Therefore, due to their aberrant activity in PDAC, the inhibition of PCs, by siRNA or chemical inhibitors, in combination with conventional treatments have been tested in vitro on 2D adherent cancer cells as well as in 3D pancreatic tumorsphere. In both cases, PCs inhibition sensitizes pancreatic cancer cells to current chemotherapy and radiotherapy. The combination treatment shows a reduction of the different tumorigenic properties of pancreatic cancer, including cell growth, motility and survival. These and other findings highlight that PCs inhibition, in combination with the current first line treatment, might be beneficial for treatment of PDAC and could improve the current therapeutic status of pancreatic cancer patients.

P44 - 3D mammary acinus model for breast cancer studies using the “Cellular Capsule Technology”

Aurélien Richard

The LP2N/BiOf (UMR5298) develops a new co-extrusion microfluidic technique designed to produce 3D encapsulated organoids and tumoroids in alginate capsule or tube. This technique, which has been patented several times and transferred to the VoxCell facility (UAR TBM Core in Bordeaux) is used for developing innovating 3D models closer to reality than flat 2D culture. In the context of oncology, these new models are much sought after because of their relevance for 1/ the study of the physiology and the microenvironment of

various cell types, and 2/ the study of the therapeutic molecules diffusion in a 3D tissue. Thanks to its biocompatibility, the alginate allows 3D cell proliferation in a confined environment where every parameter is tunable: cell types (mono/co-culture), capsule rigidity and extracellular matrix type and concentration. With this technology, at the VoxCell facility we developed a new 3D model of mammary epithelium stable for weeks. It consists of hollow capsules of approximately 200 μm with a central lumen, and a mammary epithelium attached onto the alginate inner wall via a matrix (Matrigel). In the context of breast cancer, it has been observed that the cells contained in pre-metastatic niches overexpress membrane receptors like integrin beta 3. To mimic these niches, we mixed a few mammary epithelial cells that overexpress the integrin beta 3 (MCF10A-ITGB3-GFP) with a healthy mammary epithelium (MCF10Awt). By 3D imaging (spinning disk) we observed the instability of cancerous cells in a healthy mammary epithelium under the influence of extracellular matrix modulation. Thus, this 3D encapsulated hybrid epithelium model mimics the epithelial-mesenchymal transition in pre-metastatic niches.

P46 - Desert hedgehog related endothelial dysfunction is sufficient to induce diastolic dysfunction

Paul Rouault

Introduction: Heart Failure with Preserved Ejection Fraction is proposed to be caused by endothelial dysfunction (ED) in cardiac small vessels. Objective: Our goal is to demonstrate that ED is sufficient to induce diastolic dysfunction using mice with genetically induced ED. Methods: We used Desert Hedgehog (Dhh) endothelial deficient mice (DhhECKO) as such a model. Indeed, we recently demonstrated that Dhh is critical for endothelial integrity. Practically, DhhECKO mice were administered with tamoxifen at 8 weeks of age to induce Dhh KO and ED. Mice were sacrificed one month later. Results: As expected, DhhECKO mice exhibited phenotypic changes in their cardiac small vessels characterized by a pro-inflammatory phenotype, a pro-thrombotic phenotype and abnormal endothelium permeability. Notably, these changes are sufficient to induce diastolic dysfunction since DhhECKO mice presented significantly increased end-diastolic pressures, while their left ventricular ejection fraction was comparable to control mice. Moreover, DhhECKO have a reduced exercise tolerance, suggesting a heart failure. Importantly, antiaggregants therapies prevented the occurrence of both diastolic dysfunction and exercise intolerance in these mice. Conclusion: Altogether, these results demonstrate that small vessel thrombosis may participate in the pathophysiology of diastolic dysfunction

P47 - DNASE1L3 function in obesity-mediated inflammation and metabolic syndrome

Anaïs Roubertie

Obesity is a major health disease affecting 13% of the world population. The development of metabolic syndrome and severe complications associated with obesity is attributed to the chronic low-grade inflammation that occurs in metabolic tissues such as the white adipose tissue (WAT) or the liver. Recently, circulatory self-DNA (cfDNA), which accumulates systemically in obese individuals, was shown to aberrantly activate innate immune responses and to contribute to WAT inflammation. While deoxyribonucleases (DNASES), in particular DNASE1L3, regulate cfDNAs levels, no study has yet evaluated their role in obesity. Objective. We aimed to define the role of DNASE1L3 in the regulation of cfDNA immunostimulatory potential during obesity and to assess whether its deregulation plays a role in WAT inflammation in obese subjects. Methods. Dnase1L3^{-/-} mice and controls were fed with a high fat diet (HFD) during 13 weeks. Body weight gain was measured weekly. Metabolic parameters (glycemic, lipidic and hepatic status) were evaluated after 6 and 12 weeks of diet. WAT inflammation was measured by flow cytometry. DNASE1L3 expression was also quantified in human peripheral blood mononuclear cells (PBMC) from obese individuals and compared to healthy counterparts. The analysis was completed by flow cytometry-based fluorescence in situ hybridization (Flow-FISH), using fluorescently labelled probes targeting DNASE1L3 mRNA to measure DNASE1L3 expression simultaneously in multiple cell types of the blood. Results. In a mouse model of diet induced obesity, we showed that dnase1L3 deficiency i) increase weight gain, ii) exacerbate the development of metabolic syndrome, iii) increase pro-inflammatory macrophages infiltration into WAT. In human, we showed that DNASE1L3 expression is decreased in PBMC of obese patients compared to healthy donors (mainly in B cells) while circulating self-DNA levels are increased. We next plan to elucidate the cellular and molecular mechanisms of DNASE1L3 action and its therapeutic potential in obesity. Conclusion. Characterizing DNASE1L3 function in obesity will shed light of a novel pathogenic loop that contribute to obesity-associated metabolic syndrome and lead to development of novel therapeutic tools that are critical for obese patients.

P48 - Insight into Toxoplasma gondii's basal pole: focus on new components involved in constriction and cell-cell communication

Chloé Roumégous

Apicomplexa phylum gathers several parasites responsible for severe human diseases, such as malaria (*Plasmodium falciparum*), cryptosporidiosis (*Cryptosporidium parvum*) and toxoplasmosis (*Toxoplasma gondii*). Those

single-cell parasites are highly polarized and rely on their apical complex to actively invade and egress from the host cell. At the opposite side of the parasite, the basal complex is composed of two sub-compartments, the basal ring and the posterior cup, but their molecular composition and organization remain poorly understood. In *Toxoplasma gondii*, three myosin heavy chains are important components of the basal complex, being involved in several critical steps of the lytic cycle, including motility (TgMyoC), cell division (TgMyoJ) and cell-to-cell communication (TgMyoI). In order to extend our knowledge on the basal pole organization, we performed a proximity labelling assay using TgMyoJ as a bait. Coupled to mass spectrometry, this assay provided us with several putative TgMyoJ-interacting partners and we first selected 12 of them for further analysis. Nine of those proteins have been localized at the basal pole and attributed by expansion microscopy to different sub-compartments, including two new ones, located above the basal ring and under the posterior cup. Even though none of the nine basal complex components (BCCs) are essential for parasite growth *in vitro*, we showed that BCC1 is critical for basal complex constriction, likely by interacting directly with TgMyoJ, and four others are involved in intravacuolar cell-to-cell communication, necessary for rosette organization and synchronicity of division.

P49 - Breast cancer cells transfer invasive properties through collagen migrasomes

Lucile Rouyer

Metastasis is the leading cause of breast cancer-related deaths. During this process, tumor cells acquire invasive and migratory capacities in order to invade surrounding tissues. To achieve this, the tumor microenvironment (TME) including the extracellular matrix (ECM) are altered to facilitate cancer cell proliferation and dissemination. One of the most abundant components of this ECM is collagen. Thanks to the collagen receptor, cells can attach to the ECM in order to migrate and invade. During the process cell membrane fragments are pulled out under the effect of mechanical forces. In parallel, cancer cells have the ability to produce vesicles in the TME involved in cell-cell communication and tumor progression. Here we highlight that some depositions, named collagen migrasomes, can be formed by cancer cells during migration and specifically attached along collagen fibers. Collagen migrasomes are identified by discoidin receptor 1 (DDR1) enrichment and their formation is promoted when cell-ECM interactions are increased, such as in tumor microenvironment. We characterized these collagen migrasomes, their ultrastructure as well as their molecular composition in terms of proteins and nucleic acids, showing that they are different from classical migrasomes known so far. Indeed, migrasomes have been recently discovered, they are vesicles formed at the end of retraction fibers of migrating cells on fibronectin ECM. Moreover, collagen

migrasomes are very stable structures and can be internalized by surrounding cells. After internalization, they modify the differentiation status and the phenotype of recipient cells, promoting epithelial to mesenchymal transition, matrix degradation, invasion and aggressiveness *in vivo*. Thus, we identified a function of these membrane deposits playing a role in cell-cell communication by transferring invasive properties. Consequently, cancer-related collagen migrasomes could be a new player in the tumor invasion process.

P50 - DNASE1L3 regulates the therapeutic efficacy of immunogenic chemotherapies

Pauline Santa

Detection of tumor-derived DNA (tDNA) by dendritic cells (DCs) plays a crucial role in activation of anti-tumor immunity by stimulating the production of type I interferons (IFN-I). IFN-I is associated with improved patients' outcomes and better efficacy of immunotherapies. In addition, chemotherapies and radiotherapy boost anti-tumor responses by increasing IFN-I production induced by tDNA. We have characterized a nuclease produced by DCs called DNASE1L3 that digests DNA released by dying cells and thus limits self-DNA abundance and its immunostimulatory potential. However, it remains unknown whether DNASE1L3 may be involved in the regulation of tDNA-induced anti-tumor immune responses. Given the expression profile of DNASE1L3 and its property to regulate the levels of extracellular cell free DNA, we aimed to characterize the impact of DNASE1L3 deficiency on cancer progression and responsiveness to chemotherapies, radiotherapy and immunotherapy. Dnase1l3 deficient mice were crossed with MMTV-PyMT mice that spontaneously develop mammary adenocarcinomas. In addition, transplantable orthotopic tumor models were established in Dnase1l3 deficient mice using the EO771 mammary carcinoma cell line. Tumor growth was followed weekly and the anti-tumor immune response was evaluated by flow cytometry at endpoint. Dnase1l3 deficient mice harboring either spontaneous or transplantable mammary tumors were also treated with immunogenic chemotherapies, such as Doxorubicin and Teniposide. After five consecutive treatments every other day, tumor growth was followed daily and the overall survival of mice was evaluated. Our preliminary results show that Dnase1l3 deficiency didn't directly affect the growth of either spontaneous or transplantable tumors, or the tumor immune cell infiltrate. Though, the therapeutic efficacy of the chemotherapies was strongly reduced in Dnase1l3 deficient mice. Accordingly, Dnase1l3 deficient mice succumbed faster compared to control wild type mice upon chemotherapeutic treatment. Thus, DNASE1L3 may somehow process tDNA to enhance its immunostimulatory potential. Further studies are needed, particularly of the mechanisms of action of DNASE1L3 in the regulation of anti-tumor immune responses induced by immunogenic therapies. Characterizing DNASE1L3 function in cancer may

contribute to the development of novel therapeutic strategies to boost anti-tumor immunity and the efficacy of current therapies.

*P51 - HIF-1 α implication in gastric carcinogenesis mediated by *H. pylori**

Mariana Saraiva

Context and objectives. Hypoxia is a common feature of solid cancers, involving two main factors: HIF-1 α , an oxygen-dependent subunit, and HIF-1 β . Together, they form a transcriptional complex promoting the transcription of several genes. *Helicobacter pylori* infection is the major risk factor for gastric adenocarcinoma. *H. pylori* infection affects half of the world's population and leads to gastric cancer in ~10% of cases. *H. pylori* infection leads to the "hummingbird" phenotype, epithelial-to-mesenchymal transition (EMT) and induces the emergence of cells with cancer stem cell (CSCs) properties. CSCs targeting is a challenge. Consequently, further understanding is crucial to enable therapeutic options. Previous reports indicate that *H. pylori* infection induces HIF-1 α protein expression. However, its involvement in the gastric carcinogenesis mediated by *H. pylori* has not been studied. Therefore, this project aims at determining the implication of HIF-1 α in EMT and generation of CSCs in gastric carcinogenesis. Methods. Gastric epithelial cells were silenced for HIF-1 α and co-cultured or not with *H. pylori* strains for 24h. Results. *H. pylori* infection increased the percentage of cells harbouring the hummingbird phenotype, but HIF-1 α knockdown did not affect these values. *H. pylori* infection increased the gene and protein expression of mesenchymal markers ITGB1 and Snail. HIF-1 α knockdown impacted the protein expression of both markers. Snail's nuclear translocation increased upon infection and was decreased by HIF-1 α knockdown. Infection increased the formation of tumorspheres, which decreased upon HIF-1 α knockdown. Immunofluorescence analysis for CSC markers revealed increased percentage of CD44+ cells upon infection, and a decrease in the HIF-1 α knockdown cells. Similar results were obtained with flow cytometry for CD44+ cells. Altogether, the results obtained indicate a role of HIF-1 α in the EMT process induced by *H. pylori* infection and in the emergence of CSCs. It suggests that HIF-1 α is implicated in the gastric carcinogenesis mediated by *H. pylori*

P52 - Helicobacter pylori induces hepatic lesions in mouse model of gastric carcinogenesis

Elodie Sifré

Gastric cancer is mainly caused by chronic infection with the bacterium *Helicobacter pylori* which colonizes the human stomach lifelong. It induces chronic gastritis, evolving in some cases to intestinal metaplasia, dysplasia and adenocarcinoma. *Helicobacter pylori* infection has also been associated with

extra-gastric diseases, and its role in liver pathogenesis has been suggested. This study evaluated the consequences of mice infection with different strains of gastric Helicobacters, including mouse-adapted strains of *H. pylori*, on liver pathogenesis after one year. Histopathological analysis of paraffin-embedded liver tissue sections were doubled-blindly scored for inflammation and other preneoplastic lesions. Mice infected with *H. felis* and with some strains of *H. pylori* developed more liver inflammation and steatosis, known precursor lesions of liver carcinogenesis, compared to controls. Understanding *H. pylori* infection's impact on extra-gastric lesions could in fine help detect and prevent the emergence of other digestive-track related diseases.

P53 - Establishing division of labor in a multicellular model of the yeast Saccharomyces cerevisiae

Robyn White

Multicellularity is a major evolutionary event that has occurred at least 25 times independently since the origin of life. This transition is thought to be favored by the selective advantages it confers to organisms, such as the division of labor, a mechanism of cellular specialization. However, some aspects of this transition are still relatively poorly understood: the mechanisms leading to its stabilization are not well described because they are rather complex to study in a natural context. In order to describe them, my project consists in the establishment of a synthetic multicellular model of division of labor in the snowflake of the yeast *Saccharomyces cerevisiae*. My experiments have established the feasibility of this project, by validating the three conditions necessary for the implementation of the division of labor. An experimental evolution of this system will then allow to study the mechanisms of stabilization and transmission of the division of labor.

P54 - Mutated in colon cancer protein (MCC): Role in maintaining blood brain barrier integrity through wnt signalling

Valentin Delobel

Introduction: Blood brain barrier (BBB) disruption is critical for neurological disorders physiopathology. Wnt canonical signaling regulates BBB development and its stability. We have reported that excessive activation of the ubiquitin ligase PDZRN3, a Wnt canonical signaling inhibitor, destabilizes the BBB. However, the molecular pathway is still poorly understood. Aim: We aimed to identify novel Wnt signaling partners involved in BBB stabilization. Methods: Interacting effectors were searched by proximity labeling (BioID) in brain endothelial cells (EC). We employed lentiviral vector mediated overexpression and knockdown (KD) to manipulate gene expression in EC and performed biochemical studies to investigate signaling mechanism in vitro and in vivo.

Results: MCC, a tumor suppressor gene, was identified as a PDZRN3 interactant in EC. Mcc KD impairs directed EC migration in the flow direction and decreases EC permeability. In mature EC, MCC is mainly found in the phosphorylated state dependent of the Wnt-activated Casein Kinase I ϵ (CKI ϵ). We showed that PDZRN3 stabilizes MCC expression in EC, by blocking CKI ϵ activity. During post-natal mouse BBB development, MCC undergoes a switch from un- to hyper-phosphorylated state. In mouse mutants, specific EC ectopic Pdzrn3 expression reversed MCC phosphorylation correlated with BBB destabilization. Discussion: This study discovers a Wnt-dependent post translation modification of MCC in EC, as a reversible process which dynamically regulates BBB stability.

P55 - Characterization of pathogenic mutations of the mitochondrial transmembrane GTPase MFN2

Chloe Barsa

Mitochondria are dynamic organelles that move, fuse and divide and these dynamics are essential for mitochondrial maintenance, function and degradation. In vertebrates fusion of outer mitochondrial membranes is governed by two dynamin-related GTPases anchored to the mitochondrial outer membrane: mitofusins MFN1 and MFN2. These mitofusins exert common as well as specific functions: while MFN1 and MFN2 contribute to mitochondrial fusion and mobility, MFN2 has been specifically involved in the formation and/or modulation of mitochondria-ER contacts. Despite advances in their biochemical and structural characterization, their precise mechanisms of action remain debated. Charcot-Marie-Tooth type 2A Disease (CMT2A) is a peripheral neuropathy caused by autosomal dominant mutations of MFN2. Despite improved knowledge of the disease and of the involved gene, the precise physiopathological mechanisms remain largely unknown and the disease knows no curative treatment. In this work, we characterize the functional and physiological consequences of pathogenic MFN2 mutations in Mouse Embryo Fibroblasts (MEFs) that are devoid of endogenous mitofusins (MFN1 and MFN2). This improves our understanding of the processes governed by MFN2 and provides a functional diagnosis to CMT2A patients. We find two major types of MFN2 variants linked to CMT2A: those that loose and those that maintain the capacity to mediate mitochondrial fusion. We infer that MFN2-variants unable to catalyze fusion are pathogenic and provoke disease through alteration of the mitochondrial fusion process. We still ignore whether fusion-competent MFN2-variants are pathogenic and – if – how they provoke CMT2A. Specifically, we ignore whether these variants: (1) display minor fusion defects (escaping detection with available assays and cell models) (2) affect other MFN2-functions (like mitochondrial mobility or contacts with ER). (3) alter functions that ‘are specific for’ or ‘only become limiting in’ (peripheral) neurons

P56 - Investigation in yeast of the mitochondrial DNA mutation m.9035T>C detected in patients suffering from neuromuscular diseases

Camille Charles*

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.

P57 - NETs/DNases balance in severe COVID-19 disease

Geoffrey Garcia*

Neutrophils Extracellular Traps (NETs) are web-like structure composed of DNA and proteins from neutrophil cytoplasm. They are released in a process called NETosis from neutrophil to trap pathogens in a process called immunothrombosis: NETs, with other immunological actors, activate coagulation to form a clot containing the pathogen, thus preventing its dissemination in the organism. Physiologically, they are regulated by DNases. Interestingly, An increase of NETs markers has been observed during COVID-19: excess of NETs contribute to the physiopathology of severe COVID-19 by an

uncontrolled immunothrombosis and coagulopathy. We hypothesised that a decrease of DNases activity in severe COVID-19 patients could be responsible of a NETs clearance impairment, and lead to disease progression. We developed a method to explore total DNases activity in human samples and evaluated it on 34 patients with moderate and severe COVID-19 included in COLCOV19 protocol. We measured two NETosis markers: citrullinated histone H3 (H3cit) and DNA-H3cit complex. We found that our fluorimetric approach to explore total DNases activity in human sample is an easy, repeatable and reproducible method, and we define pre-analytical conditions to perform it. Our results showed that patients with severe symptoms have an increase of NETosis markers compared to mild patients, as described in the literature. In addition, total DNases activity is increased in severe patients. However, NETosis markers/total DNases activity ratio is increased in severe patients, showing an unbalanced regulation of NETs. We believe that DNases activity is not increased enough to limit NET elevation in human plasma during severe COVID-19 and lead to an aggravation of the disease by an impairment of NETs clearance. Our work confirms the importance of NETosis and DNases implication during severe COVID-19 and leads to explore and understand the mechanism of DNases dysregulation in this disease.

P58 - Looking at the memory response in normal appearing skin of vitiligo
Laure Migayron*

Resident memory T cells (TRM) are a critical component of the tissue immune response but are also implicated in the physiopathology of cutaneous chronic inflammatory diseases, such as vitiligo. In this depigmenting disease characterized by the loss of epidermal melanocytes, the involvement of skin TRM with a type-1 skewed immune response (production of IFN γ and TNF α) in lesions is commonly admitted. Our recent data suggest an additional role of a type-2 immune response with local production of IL-13. In addition, increasing evidence suggest that non-lesional (NL) skin in cutaneous inflammatory diseases like psoriasis or atopic dermatitis already display an altered immune response. Yet, little is known regarding the T cell immune response NL skin of vitiligo patients. We aimed to decipher the phenotype and function of the TRM infiltrating normal appearing skin of vitiligo patients. While infiltration of CD8 T cells was more prominent in vitiligo perilesional (PL) skin, NL and PL skin displayed similar subsets of TRM defined by the markers CD69, CD103 and CD49a. However, skin T cells isolated from PL vitiligo skin showed a decreased expression of PD-1 which may be responsible of a breakdown of a quiescent state of TRM in NL areas. Interestingly, following ex vivo T cell activation, NL skin displayed an intermediate inflammatory transcriptional profile compared to healthy skin and PL vitiligo skin. In addition, activated NL vitiligo skin T cells produced both type-1 and type-2 related cytokines (including IL-13), albeit

at lower levels than activated vitiligo PL skin T cells. Interestingly, IL-13 was implicated in the inflammatory response of epidermal cells by inducing CCL18 production. Taken together, our results suggest that “pathogenic” TRM are already present in pigmented areas in a quiescent state and could contribute to inflammatory flare-ups and relapse of vitiligo.

P59 - Involvement of Reptin in invadosome formation and tumor invasion

Mélanie Moreau*

Metastasis formation is the main cause of cancer related death. They are the consequence of tumor invasion that is the ability of cancer cells to colonize new tissue. To do so, cells must migrate across anatomical barriers, notably by degrading the extracellular matrix (ECM). This ability is conferred by invadosomes, which are membrane protrusions composed of F-actin structures associated with MMPs activity. In a previous study, we used an approach combining laser microdissection and mass spectrometry analysis to define the invadosome proteome in the NiH3T3-SrcY527F cell model. These cells overexpress a constitutively active form of Src protein promoting rosette invadosome formation. This approach revealed that Reptin is 6 times enriched in invadosomes in comparison with the total cell lysate. Reptin is a AAA+-ATPase involved in different cellular functions including DNA repair, replication and molecular co-chaperoning complexes. Reptin is a member of the R2TP complex, which is required for the assembly and conformation of many protein complexes. We demonstrated that Reptin, as well as the other members of the R2TP complex (Pontin, RPAP3 and PiH1D1), co-localize with rosette invadosomes. By a siRNA approach we have shown that Reptin depletion significantly decrease the NiH3T3-SrcY527F ability to form invadosomes and to degrade the ECM. Moreover, in Reptin depleted cells, we noticed a recover of the wildtype phenotype characterized by the presence of stress fibers and the absence of invadosomes. That point reflects a loss of SrcY527F activity suggesting a molecular link between Src and Reptin. We confirmed this hypothesis by showing a Reptin and Src co-localization and by highlighting a decrease of Src-Tyr419 phosphorylation state in Reptin depleted cells without affecting its total expression level. To identify the molecular mechanism involved in the modulation of Reptin-dependent Src activity, we use bibliographic and exploratory proteomic approaches to determine proteomic profiles with or without Reptin and Reptin partners and their major functions in invadosomes. Therefore, we revealed the involvement of autophagy in the regulation of proteins, such as Fak, in a Reptin-dependent manner. Taken together these results will allow a better understanding in the mechanism of invadosome formation mediated by Reptin and the R2TP complex. This work will allow a better comprehension of mechanisms involved in the process of tumor invasion.

P60 - Effect of proprotein convertases inhibition on Cancer Stem Cells tumorigenic and invasive properties in gastric adenocarcinoma

Anissa Zaafour*

Proprotein convertases (PCs) are enzymes involved in the maturation of numerous precursor proteins implicated in fundamental cellular processes: proliferation, survival, adhesion, invasion, immunity... The role of PCs in tumorigenesis has been extensively studied: it contributes to tumor progression in many malignancies such as rhabdomyosarcoma, colon carcinoma, and others. However, the involvement of PCs in gastric adenocarcinoma (GC) tumorigenesis has been poorly studied until now and need to be investigated. GC is the fourth leading cause of cancer-related death worldwide, it's very often detected at an advanced stage and associated with poor prognosis and high risk of relapse. This can be explained by the presence of cancer stem cells (CSCs), a subpopulation of cancer cells able to self-renew, differentiate, initiate tumor growth, metastasize, resist to conventional therapies, and trigger cancer relapse. CSCs hold their properties and survival through hijacked signalling pathways such as the Hippo pathway as it has been demonstrated in GC. They possess an epithelial to mesenchymal transition (EMT) signature reflecting cancer aggressiveness. Use of decanoyl-RVKKR-chloromethylketone (CMK), general chemical PCs inhibitor, in four different GC cell lines, allowed to highlight that PCs inhibition decreased GC CSCs ability to initiate tumorspheres, sustain tumorspheres growth, as well as their drug efflux capacities. It also reduced the transcriptional activity of downstream YAP/TAZ/TEAD oncogenic effectors of the Hippo pathway, suggesting CMK could inhibit CSCs properties via YAP/TAZ/TEAD activity. Moreover, the invasiveness of GC cell lines was highly impaired by PCs inhibition. This effect was associated to a decrease of expression of some invasive and mesenchymal markers and of EMT transcription factors nuclear expression: ZEB1 and Snail. To conclude, PCs inhibition seems to be a potential strategy to target CSCs in GC. Further investigations are required to refine this strategy and better understand the molecular mechanisms implicated in anti-CSC effects in GC.

P61 - Identification of TREM1+ CD163+ myeloid cells as entropic immunosuppressive cells that associate with poor survival

Domitille Chalopin-Fillot*

Hepatocellular carcinoma (HCC) is an inflammation-associated cancer arising from viral and non-viral etiologies. Immune checkpoint blockade primarily benefits patients with viral HCC. Expansion of suppressive myeloid cells is a hallmark of chronic inflammation and cancer, but their heterogeneity in HCC is not fully resolved and might underlie immunotherapy resistance in the steatohepatitis setting. Here, we present a high resolution atlas of hepatic innate immune cells (~100,000 single-cell transcriptomes) from patients with HCC and unravel several discrete populations of entropic monocytes and dendritic cells that expand in the tumoral tissue. Among them, we identified a population of suppressive THBS1-Monocyte that is rare in viral HCC but expands in the steatohepatitis setting. These THBS1-Monocytes dually express granulocyte- and macrophage-lineage genes and are selectively marked by elevated expression of TREM1 and CD163. They most potently suppress T cell activity *ex vivo*, highly express TGF β and are spatially enriched with FAP+ fibroblasts at HCC fibrotic lesions. The density of THBS1-Monocytes significantly correlates with advanced grades HCC and poor patient survival, and associates with resistance to immune checkpoint blockade in other solid tumors. The expression of TREM1 alone confers poor prognosis in HCC and blockade of its signaling in mice promotes tumor eradication. Our data support myeloid subset-targeted immunotherapies via TREM1 to treat HCC.

P62 - A novel role for the Adenovirus protease during virus entry

Jean-Baptiste Vergnes*

Adenoviruses are non-enveloped viruses that enter the cell by endocytosis and escape from the endosomal compartment. To cross the endosomal membrane the capsid undergoes partial disassembly and releases capsid protein VI inside the endosome. Protein VI, with its amphipathic helix, binds and damages membranes. Cytosolic virion will then be transported to the nucleus to initiate viral gene expression and replication. It remains unclear how the partial disassembly allows protein VI release. Possible mechanisms include acidification inside the endosome and/or mechanical disruption induced by the binding of the virion to cell receptors. The Adenovirus protease, a sequence-independent DNA binding protein incorporated inside the virion, is processing different proteins of the Adenovirus through a one dimensional biochemical reaction. This step called maturation is happening after the virus assembly and is essential for the virus infectivity. Indeed, a mutant with unprocessed proteins remains stable inside the endosome, thus there is no release of protein VI and no membrane damages. Consequently, with unprocessed proteins, the

Adenovirus is unable to escape the endosome. We produced and purified the Adenovirus protease in order to reconstitute the maturation process in vitro to better understand its role in the viral cycle. We observed an unexpected cleavage that could be a trigger in the virus partial disassembly. Our data thus indicate that the protease may have so far unrecognised functions during virus entry steps.

P63 - Interplay between the phosphatase PRL2 and Tumor Microenvironment Promotes Glioblastoma Progression

Tiffanie Chouleur*

Protein Tyrosine Phosphatases (PTPs) are involved in oncogenesis in several types of cancer. Still, their exact roles in glioblastoma (GBM), the most aggressive type of primary brain tumor, remain elusive. Here we report that the PTP PRL2 (PTP4A2) is associated with a poor prognosis in gliomas, and its expression correlates with GBM aggressiveness. Using GBM spheroids, we demonstrate that PRL2 over-expression promotes tumor growth and reduced mouse survival rate in an orthotopic xenograft model. In contrast, the knockout of PTP4A2 leads to reduced tumor growth and increased apoptosis, which is linked to a rise in pro-inflammatory signaling. In vitro assays show that cell proliferation was not affected in PTP4A2 deficient or overexpressing cells highlighting the importance of the microenvironment in PRL2 functions. Finally, pharmacological inhibition of the PRLs using JMS-053 inhibits GBM cell viability and spheroids growth. Collectively, our results indicate that PRL2 promotes GBM growth in response to microenvironmental pressure and support that the targeting of PRL2 opens new avenue for therapeutic strategy in GBM treatment.

PLATFORMS

Vect'UB : Vectorology expertise at the service of research

Véronique Guyonnet-Dupérat

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. The most important step in viral vector production is the proper design of vector. 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, we can also help you to design, construct and product the viral vector containing your gene of interest, or CRISPR tool sequences. 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. 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!

Nano luciferase a new tool for in vivo bioluminescence imaging available at the VIVOPTIC platform.

Coralie Genevois

Bioluminescence imaging (BLI) is a technique with a low background noise and high sensitivity. The ATP-dependent intracellular firefly luciferase (Fluc) is currently the most widely used luciferase for BLI. Recently, a new ATP-independent enzyme from the deep sea shrimp *Oplophorus gracilirostris* called NanoLuc (Nluc) and its specific substrate furimazin were engineered. Nluc is a small luciferase of 19 kDa (easy to clone including in viruses) which is brighter than any luciferases. Nluc proteins are available in an un-fused nonsecreted form (Nluc), a secreted form (secNluc) and a short half-life form (NlucP); Nluc can also be used for fusion proteins. Nluc produce light at a wavelength of 460 nm that is not optimal for the near infrared (NIR) window (700–900 nm) that defines the spectral zone for minimal photon absorption by living tissues but Nluc is so bright that it can be used to detect signals in deep tissues such as brain tumors and metastases. As Nluc and Fluc were using different substrate,

they can be detected sequentially. Up to now, only Nluc substrate furimazin was commercially available as Nano-Glo™ not optimal for in vivo use. A new substrate called Nano-Glo® In Vivo Substrate optimized for in vivo application has been developed by Promega and tested in prime at VIVOPTIC. It provide bright signal from tumors including in deep locations. Images are shown on the poster. VIVOPTIC can help you to adopt Nluc for your project. absorption by living tissues but Nluc is so bright that it can be used to detect signals in deep tissues such as brain tumors and metastases. As Nluc and Fluc were using different substrate, they can be detected sequentially. Up to now, only Nluc substrate furimazin was commercially available as Nano-Glo™ not optimal for in vivo use. A new substrate called Nano-Glo® In Vivo Substrate optimized for in vivo application has been developed by Promega and tested in prime at VIVOPTIC. It provide bright signal from tumors including in deep locations. Images are shown on the poster. VIVOPTIC can help you to adopt Nluc for your project.

Oncoprot, mass spectrometry proteomic profiling platform

Anne-Aurélie Raymond

The Oncoprot platform has developed and proposes an innovative proteomic analysis method adapted to fixed material (FFPE cells and tissues). The Oncoprot analytical procedure (patent BNT219885FR00, 2015 extended to Europe and USA in 2017, patent BNT230417EP00, 2021) is based on the combined use of two approaches: laser microdissection and proteomic analysis by mass spectrometry. A cell, a subcellular compartment (Ezzoukhry Z et al. Nature Communications, 2018) or a fixed tissue is first microdissected. The cross-linking due to fixation is reversed and proteins are extracted. Proteomic analysis is then performed using a next generation high-resolution mass spectrometer. Thus, while conventional immunohistochemistry analyses without quantification the expression profile of a single or a limited number of proteins (by multiplexing) per histological section, the Oncoprot technology allows an optimized use of these samples by identifying and quantifying several thousands of proteins from very small quantities of material (1mm² tissue on a 5µm thick section). Thanks to this approach, we have identified new biomarkers of clinical interest (Henriet et al, Hepatology, 2017), one of which is already used in clinical routine at the Bordeaux University Hospital for the diagnosis of benign hepatic tumors at risk of bleeding (Sala et al, Hepatology Communications, 2020). In addition, we have developed a machine learning tool that uses the entire proteomic profile as a new tool for diagnosis, prognostic assessment or prediction of treatment response and can improve patient management (Dourthe et al, Hepatology, 2021). This unique procedure is very interesting for translational and clinical research and paves the way for precision medicine. Our expertise in exploratory proteomic analysis allows

us to help you in your projects of protein expression analysis from cellular or tissue models, from the conception of the analytical design to the bioinformatic and statistical processing of the data. Thanks to dedicated bioinformatics and integrative biology tools and the intervention of our team's bioinformatician, we can provide support in the biological interpretation of proteomic data and in the graphical representation of your results.

CRISP'edit core facility

Valérie Prouzet-Mauléon

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

Cytométrie Bordeaux FACSility

Vincent Pitard

Cytométrie Bordeaux FACSility propose une gamme de cytomètres en flux pour l'analyse et le tri de vos suspensions cellulaires et particulaires

Mise à disposition des équipements après formation

Étude de faisabilité

Collaboration scientifique

Tarifs : Facturation à la minute du temps consommé

Spécificités : Trieur sous PSM, large choix d'analyseurs et de configurations optiques

Qualité : Réservation en ligne des instruments, serveur de stockage des données, 4 postes d'analyse Diva / Flowjo, service accessible 24H/24

Plateforme Universitaire Laboratoire L3

Patricia Recordon-Pinson

Une structure adaptée aux manipulations en confinement de niveau 3 (rétrovirus, bactéries et parasites) est à votre disposition au sein de l'Université de Bordeaux. Nous pouvons vous former ou vous accompagner sur de nombreux projets. Nous répondons à des demandes récurrentes de collaborations qui permettent de développer des programmes de recherche translationnelle en partenariat avec des équipes de compétences complémentaires aux nôtres que ce soit en biophysique, chimie ou biologie. Dans le domaine de la virologie, certains de ces projets étudient la réplication du VIH-1, la résistance aux antiviraux ou encore la recherche d'inhibiteurs de l'infection VIH. Nous avons pu également mener à bien des contrats en partenariat avec l'industrie. De nombreux projets de recherche en collaboration avec différents laboratoires académiques locaux et nationaux ou des entreprises privées concernent le SARS-CoV-2. Sont également réalisés en L3, la manipulation d'oncogènes pour la création de lignées immortalisées. De plus, la manipulation de banques CRISPR, une technologie maintenant incontournable pour le développement de la recherche thérapeutique permettant l'editing des génomes, se fait également en confinement L3. Plus récemment, le séquençage nouvelle génération fait partie des outils disponibles au sein de la plateforme. Notre perspective est d'utiliser le savoir-faire de la plateforme, notamment pour l'évaluation de molécules ou banques d'inhibiteurs, pour élargir les modèles disponibles à d'autres virus à ARN émergents, comme le Zika, la Dengue ou le Chikungunya.

CellOxia Core Facility : Modeling the hypoxic niche

Arnaud Villacreces

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 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.

OneCell Platform: RT-PCR & Single Cell Libraries

Xavier Gauthereau

Platform offers : a full service, technical advice and support for users an automated approach for nucleic acid qualification an automated approach for real time PCR a Next GEM Technology for Single Cell Gene Expression Platform services: Qualification of nucleic acids (RNA/DNA Integrity Number) Panels and primer design, qRT-PCR Automated preparation of 384 well qPCR plates Analysis of relative and absolute gene expression levels Library construction for Single Cell analysis 10x Genomics Collaboration with UB'Facility/Bordeaux for Cell Sorting, with Get-Plage/Toulouse and PGTB/Cestas for sequencing, with CBiB/ Bordeaux for DATA analysis.

VoxCell « The Cellular Capsule Technology » A 3D cell model facility

Laetitia Andrique

The VoxCell Facility generate 3D cell model on demand with the Cellular Capsule Technology. This interdisciplinary facility combines a microfluidic technique with Biology to generate 3D organoids and spheroids. The platform produces alginate spherical hollow capsules containing mono-cell culture or more complexes co-cultures that can mimic the heterogeneity of the tumor niche with fibroblasts, immune cells, cancer cells, and extracellular matrix (ECM). The technique is inherently high throughput since 300,000 capsules are produced per min, and subsequently as many spheroids/organoids. Besides this high throughput format specificity, the capsule can also be seen as a closed elastic compartment. When capsules fill up the capsule, corresponding to 3D confluence, further capsule dilation leads to organoid compression. Mechanically-induced alterations of the cell response, such as proliferation, migration can thus be investigated quantitatively (Alessandri 2013, PNAS). Moreover, the alginate capsule can be customized to vary geometrical (eg. size of the capsule or the cell-containing core), mechanical (eg. stiffness and adhesive) parameters (ECM coating and nature of the ECM components) and then reproduces the forces sensed by the cells inside a solid tumor. Finally, the permeability of the alginate shell allows free diffusion of small molecules and proteins, thus permitting easy implementation of drug screening assays. Contact VoxCell : Laetitia.andrique@u-bordeaux.fr

Plateforme d'histopathologie

Nathalie Dugot-Senant et Thomas Daubon

La plateforme d'histopathologie de TBMCore vous propose : Formation aux différents appareils et mise à disposition des appareils suivants: Imprégnation en paraffine Banc d'inclusion Coupes microtomes Coupes Cryostat Automate pour colorations histologiques Automate pour IHC et IF Microscope briedfield avec Caméra Microscope confocal SP5 Leica
Prestations sur demande

PhenOtypic scrEening TechnICal platform (POETIC)

Inès Villamor

The ARTiSt group has set up a PhenOtypic scrEening TechnICal (POETIC) platform which allows us the automatization of miniaturized phenotypic screening assays, to understand the molecular mechanisms that regulate cell transformation and tumour growth. The POETIC platform all the equipment, software and support needed to facilitate a broad spectrum of screening projects. Whether you would like to screen the whole genome or simply automate image acquisition, we have fully automated systems available. POETIC is arranged into three main sections: 1. The JANUS automated liquid handling workstation (PerkinElmer) capable of si/sh/CRISPR or compound addition as well as automated cell seeding, medium changes, cell fixation and staining in 96 or 384 well formats. 2. The EnVision Plate Reader (PerkinElmer) for ELISA, Luminescence, Fluorescence Assays. 3. Automated high throughput image acquisition using Cytation™3 imaging system (BioTek) which is a cell imaging multi-mode microplate reader that combines automated digital microscopy and conventional microplate detection.

Centre de Bioinformatique de Bordeaux (CBiB) : une plateforme de soutien à la recherche, spécialisée dans l'analyse de données biologiques et médicales

Aurélien Barre

Le CBiB est spécialisé dans l'analyse de données -omiques et propose l'accès à des logiciels et des bases de données. Il possède une expertise en programmation et en l'intelligence artificielle qu'il met au service de projets de développements à façon. Le rôle des bioinformaticiens du CBiB est d'accompagner les chercheurs dans l'interprétation de leurs données. L'offre de prestation du CBiB ne se limite pas à la mise en œuvre de méthodes d'analyses de données omiques, mais s'étend du conseil en amont de la production des données jusqu'à la rédaction de méthodes bioinformatiques dans des articles scientifiques.

XenUB: La plateforme aquatique de l'Université Bordeaux

Sandrine Fedou, Nadine Thézé-Thiébaud, Hamid Rezvani et Pierre Thiébaud

Adossée à l'unité INSERM U1312, la plateforme XenUB a pour but de fournir à la communauté scientifique la possibilité d'utiliser pour leur projet de recherche le modèle aquatique amphibien xénope. Labellisée par la Fondation des Maladies Rares, la plateforme permet des expérimentations sur l'embryon et la modélisation d'un très large spectre de maladies humaines telles que les anomalies du développement et les syndromes malformatifs, les maladies génétiques liées au développement du cœur, du rein, des muscles ou encore les rétinopathies. Du fait de la très grande conservation des voies de signalisation entre xénope et l'homme, le modèle est parfaitement adapté à l'étude de la régulation de ces voies au cours de la différenciation et de l'organogenèse.

Plusieurs projets sont développés sur la plateforme dont la modélisation des maladies génétiques Xeroderma pigmentosum et l'albinisme oculo-cutané. D'autres projets consistent en la production d'embryons transgéniques exprimant des molécules de signalisation ou des marqueurs fluorescents dans le foie embryonnaire (modélisation de l'hépatoblastome) ou les neurones moteurs.

L'inactivation des gènes par la technique CRISPR-Cas9 a été mise en place sur la plateforme et permet de répondre aux demandes de projets concernant l'inactivation ciblée de gènes. Le modèle xénope permet de réaliser des criblages pharmacologiques et il constitue une alternative au modèle murin.

Aquiderm, la cellule de transfert de technologie au service de la peau

Jérôme Rambert

Créée en 2014, Aquiderm est la cellule de transfert de technologie de l'Unité BRIC U1312 et de l'Unité ImmunoConcEpt, et est rattachée administrativement à l'ADERA et l'Université de Bordeaux. Spécialisée dans le domaine de la peau, Aquiderm travaille sur des modèles pathologiques cutanés tels que le psoriasis, le vitiligo, la dermatite atopique, mais également des tissus sains, et réalise du transfert de technologie vers le tissu industriel local, national ou international.

Cette cellule de transfert propose son expertise et son savoir-faire à des sociétés de cosmétiques privées en travaillant sur des peaux saines dans le but de démontrer les allégations de ces produits en termes de soins anti-âge, protection solaire, anti-vieillessement.

Proposant des modèles cellulaires, Aquiderm réalise des cultures primaires (kératinocytes, mélanocytes, fibroblastes, cellules endothéliales, PBMC...), mais travaille aussi sur des modèles 3D (épidermes humains reconstruits (pigmentés ou non), explants de peau).

La cellule de transfert effectue des analyses protéomiques et transcriptomiques, de plus, elle est dotée d'une importante plateforme d'histologie lui permettant d'analyser tout type de tissus.

Hébergée à l'Université de Bordeaux, Aquiderm bénéficie d'un environnement scientifique lui permettant de collaborer avec d'autres cellules de transfert ainsi qu'avec des plateformes du TBMCore telles que Vect'UB ou encore CellOxia.

Aquiderm est accréditée Expert Crédit Impôt Recherche permettant à ses clients de bénéficier d'un crédit d'impôt sur les prestations réalisées par la cellule.

Metabolic Analyses Service

Benoît Pinson

You want to.... :Develop a metabolomic project, Measure the metabolite content of your biological samples , Interpret your metabolic and/or metabolomic data, Be informed in practical and/or scientific aspects of metabolic data analyses. The metabolic analyses service propose to : Guide you in your metabolic project design, Provide quantitative, reproducible metabolic data for hydrosoluble metabolites, Assist you in the interpretation of your metabolic data and to train you in diverse scientific and technical aspects of metabolic analyses

