SBM DEPARTMENT DAY

December 5th 2024
Domaine du Haut-Carré
Talence

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

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

Two guest speakers, Virginie Courtier-Orgogozo and Stephan Güttinger, will present their work during the day.

The best flash poster and oral communication will be awarded prizes by the members of the jury at the end of the day.

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

The organisers would like to thank Drs Eloïse Bertiaux, Philippe Brunet de la Grange, Audrey Burban, Ahmad Sharanek, Jean-Max Pasquet, François Moisan, Karine Frenal, Emmanuel Tetaud, Stéphane Duvezin-Caubet, Dorothée Duluc, Rigoulet Michel, Aksam Merched, Candice Chapouly, Loïc Rivière, Virginie Dinet, and Laura Desbourdes for their help in evaluating the PhD posters.

We wish you all a wonderful Biological Sciences Department Day.

Melina Abdou, Adrien Birot, Célia Bourguignon, Vanessa Desplat, Bérénice Dugue, Julie Giraud, Fridolin Gross, Nadège Hénin, Hande Sena Kandemir, Nabil Nicolas, Alexandra Prévot, Aurélien Richard.

WIFI

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Programme

Morning session

8h30-8h55 **Welcome & Registration**

8h55-9h05 **Introduction**

Thierry Noël | *Director of SBM*

9h05-9h10 **CASDEN Presentation**

Isabelle Lefebvre

9h10-9h30 **Platform presentation**

Chair: Béatrice Turcq

Benoit Pinson | *TBMCore*

9h30-10h15 **First keynote speaker**

Chair: Sophie Javerzat

Virginie Courtier-Orgogozo | *CNRS, Institut Jacques Monod*
«Using genetics to understand evolution, past and future»

10h15-10h45 **Poster Flash Talks**

Chair: Grégoire Manaud

Chloé Avril | *Immunoconcept, U5164*
«Regulation of resident memory T cell activation by immune checkpoint receptors in pigmented skin of vitiligo patients»

Alicia Defay-Stinat | *MRGM, U1211*
«Characterization of gene regulatory elements to improve our understanding of albinism pathophysiology»

Aliki Vasilakou | *Immunoconcept, U5164*
«The role of DNASE1L3 in cancer immunity»

Michel Autin | *MFP, U5234*
«Interplay between HIV-1 integration and transcription»

Narda Ardda | *IBGC, U5095*
«Towards a molecular understanding of the role of tmem70 in the biogenesis of the proton channel of f1fo-atp synthase»

Arthur Orieux | *BMC, U1034*
«Mechanisms of hypertension lesions: microvascular damage and role of inflammation»

Justine Vaché | *BRIC, U1312*
«A new chemokine identified as a key player of tumor immune microenvironment remodeling in B-catenin mutated hepatocellular carcinoma»

10h45-11h15 **First poster session & Coffee break**

11h15-12h30 **First scientific session**
Chairs: Lucie Brisson & Mathieu Metifiot

Margaux Laisné | *BMC, U1034*
«Astrocytic serglycin exhibits ambivalent properties in the control of astrogliosis in the pathophysiology of multiple sclerosis»

Arthur Poulet | *BRIC, U1312*
«Exploring Nat8L involvement in HSC self-renewal/expansion processes upon stress»

Elina Mercier | *MRGM, U1211*
«OCA2 exon 10 splicing: of mice and men»

Aurélien Richard | *TBMCore*
«3D in vitro models of the normal human breast and breast cancer»

Chloé Lambert | *MFP, U5234*
«BILBO1, BILBO2 and companions: more than a trilogy?»

12h30-13h30 **Lunch break**

Programme

Afternoon session

13h30-14h00 Second poster session

14h00-14h45 Second keynote speaker

Chairs: Jan Pieter Konsman & Manuel Rojo

Stephan Güttinger | *University of Exeter, UK*
«Replicability is not a good standard for research quality»

14h45-15h15 Second scientific session

Chairs: Jan Pieter Konsman & Manuel Rojo

Delphine Fessart | *BRIC, U1312*
«Gynaecological Cancers: Ageing, Proteostasis Dysfunction, and Resistance Mechanisms»

Adrien Birot | *IBGC, U5095*
«Unraveling the regulation of cohesin through phosphorylation»

15h15-15h40 Sustainable development session

15h40-16h10 Third poster session & Coffee break

16h10-16h55 Third scientific session

Chairs: Marija Vlaski-Lafarge & Vanja Sisirak

Audrey Burban | *IBGC, U5095*
«Targeting NLE1 signaling in glioblastoma»

Asen Daskalov | *Immunoconcept, U5164*
«Regulated cell death in fungi from a comparative immunology perspective»

Eloise Bertiaux | *MFP, U5234*
«Deciphering the role of the luminal distal proteins in the centriole elongation and architecture»

17h00 Award ceremony & closing remarks

Julie Giraud & Fridolin Gross

First keynote speaker

Chair: Sophie Javerzat

Virginie Courtier-Orgogozo | *CNRS, Institut Jacques Monod*

«Using genetics to understand evolution, past and future»

The great diversity of living forms is fascinating, and raises questions about how it came to be. How and why do specific characteristics emerge in the course of evolution? Are there general rules underlying evolution? One hundred years after the publication of Charles Darwin's opus "The Origin of Species", the structure of DNA has been elucidated. However, to this day, we still lack a general understanding of the genes and the specific mutations that contribute to evolution. I will synthesise what has been learnt in the last 20 years about the genes and the specific mutations that contribute to evolution, and how this affects our broad understanding of life on Earth.

Biography:

Virginie Courtier-Orgogozo, a former student at the École normale supérieure and agrégée in life and earth sciences, is a research director at the CNRS, head of a research team at the Institut Jacques Monod in Paris, lecturer at the École polytechnique, CNRS bronze medal, Irène-Joliot-Curie prize for "Young woman scientist" in 2014.

She is interested in the mechanisms involved in the evolution of species, in order to better understand our origins and the future of living species. In particular, she studies the evolution of bioadhesives produced by Drosophila flies to stick to various substrates during metamorphosis. Together with Arnaud Martin www.gephebase.org,she has created a database of genes and mutations that have so far been identified by the international scientific community as being responsible for natural morphological, physiological or behavioral differences in plants and animals. This compilation has revealed that evolution often passes through a limited number of genetic pathways, indicating that evolution is partly predictable.

Poster Flash Talks

Chair: Grégoire Manaud

P8* - Regulation of resident memory T cell activation by immune checkpoint receptors in pigmented skin of vitiligo patients

Chloé Avril | Immunoconcept, U5164

Vitiligo is an inflammatory depigmenting skin disease with a worldwide prevalence of 0.5 to 1%. The selective loss of epidermal melanocytes, resulting in the appearance of white patches, is the consequence of an aberrant immune response mediated by CD8 resident memory T cells (TRM) infiltrating vitiligo peri-lesional skin (border of the lesion). Results from our team suggest that self-reactive TRM cells are already present in clinically healthy pigmented skin (non-lesional) of patients and may be responsible for flare-ups and the development of new lesions. These TRM may be maintained in a quiescent / anergic state owing to the presence of immunoregulatory signals, including immune checkpoint receptors (ICRs). My doctoral project aims to assess the expression of co-inhibitory receptors by TRM cells infiltrating the non-lesional skin of patients with vitiligo, and to analyze the regulation of their expression in response to environmental stress. To this end, we first compared by spectral flow cytometry the expression of co-inhibitory receptors (PD-1, TIM-3, TIGIT, CTLA-4) and their ligands by immune, epidermal (keratinocytes, melanocytes), and dermal cells (fibroblasts) in non-lesional and perilesional skin of vitiligo patients, and healthy skin. We show that PD-1 was the main ICR expressed by TRM in the skin and that the frequency of vitiligo skin CD8 T cells expressing PD-1 was higher in the non-lesional area. In addition, the frequency of PD-L1expressing dendritic cells and epidermal cells was higher in non-lesional skin of patients, suggesting that the skin microenvironment attempts to maintain the quiescent state of TRM cells in pigmented areas. Ongoing studies aim to assess whether the modulation of the microenvironment in response to stress regulates the expression of ICRs by TRM in non-lesional skin of vitiligo patients to reveal their pathogenic potential and contribute to depigmentation.

P13* - Characterization of gene regulatory elements to improve our understanding of albinism pathophysiology

Alicia Defay-Stinat | *MRGM, U1211*

Albinism is a genetically and clinically heterogeneous disease, characterized by hypopigmentation of the skin, hair and eyes as well as visual impairment. So far, twenty-one genes are identified that are associated with oculocutaneous (OCA), ocular (OA) or syndromic (HPS, CHS) forms of the disease. Molecular diagnosis is essential to guarantee adequate medical support and genetic counselling. However, thirty percent of patients remain without molecular diagnosis after target gene sequencing. To improve the diagnosis, variants in non-coding regions are investigated, and in particular that of candidate regulatory elements. A 3D genome exploratory experiment is conducted using capture-HiC on melanocytes and retinal pigmented epithelium cells. The aim is to identify regulatory elements, i.e. promoters or enhancers, involved in transcriptional control of seven selected albinism genes (TYR, TYRP1, DCT, OCA2, SLC45A2, SLC24A2, GPR143). The strategy specifically targets regulatory elements involved in the melanogenesis pathway. The sequences of patients are investigated to look for rare variants, in trans of a pathogenic variant, located in the candidate regulatory elements identified via capture-HiC or ENCODE consortium annotation. Candidate regulatory elements

that contain a variant are characterized with a dual luciferase reporter assay system. This in vitro system allows to measure the bioluminescence emitted by the luciferase placed under control of candidate regulatory elements. If a regulatory activity is confirmed, the impact of the variant can be assessed. The first results show that this system was able to detect an increased gene expression induced by a known OCA2 enhancer, although detection of regulatory activity can be difficult, especially for distal enhancers. We propose an optimisation of the dual luciferase system. This work aims to identify new regions of interest for the molecular diagnosis of albinism.

P45* - The role of DNASE1L3 in cancer immunity

Aliki Vasilakou | Immunoconcept, U5164

Tumor DNA plays a key role in triggering anti-tumor immune responses through the stimulation of dendritic cells (DCs). Upon tumor DNA sensing through TLR9 or cGAS, DCs secrete type I interferons (IFN-I) which are essential for activating cytotoxic T lymphocytes that target and ultimately eliminate tumors. Chemotherapy (CT) and radiotherapy (RT) are known to enhance the release of tumor DNA, boosting its capacity to activate anti-tumor immunity and promote T cell-mediated tumor regression. However, the mechanisms controlling the immunostimulatory potential of tumor DNA remain unclear. We identified DNASE1L3, an endonuclease produced by DCs that degrades DNA from dying cells, limiting immune activation. As its role in cancer is still poorly explored, we developed Dnase1l3-deficient mice with spontaneous or orthotopic mammary tumors. While the absence of DNASE1L3 did not affect tumor growth, it significantly reduced the efficacy of immunostimulatory CT, suggesting that DNASE1L3 is crucial for optimal anti-tumor immunity during CT. Moreover, we observed that DNASE1L3 fragments tumor DNA released after CT. We therefore hypothesize that DNASE1L3-mediated fragmentation of tumor DNA might be crucial for enhancing its recognition by immune cells such as DCs. Accordingly, adding DNASE1L3 to CT-treated tumor cell supernatant increased its capacity to activate TLR9 and/or cGAS signaling pathway. Furthermore, our preliminary data suggest that DNASE1L3 may also regulate IFN-I production by DCs in response to tumor DNA. In fact, Dnase1l3-deficient DCs, or human DCs treated with a DNASE1L3 inhibitor, showed impaired IFN-I secretion compared to their wild-type or untreated counterparts in response to synthetic DNA stimulating TLR9. These findings highlight a critical role of DNASE1L3 in DC function and tumor immunity. We are currently exploring the underlying mechanisms involved in these processes.

P7* - Interplay between HIV-1 integration and transcription

Michel Autin | *MFP, U5234*

The host chromatin invasion by retrovirus relies on the stable integration of the viral genome into infected cell chromosomes. This integration is catalyzed by the intasome, a complex formed by the retroviral integrase (IN) enzyme and viral DNA. HIV-1 IN not only plays a role in the integration process but has additional functions, such as binding to genomic viral RNA and assisting in virion assembly. Furthermore, recent studies have reported that after integration, IN may remain associated with the integration site and that punctual mutations in the carboxyl-terminal domain known to bind RNA affect IN stability and viral gene transcription (Shelby and Goff, 2020). More recently, it has been shown that the binding of IN to TAR

RNA induces structural changes in both IN and TAR, enhancing Tat binding and potentially optimizing the transactivation of viral DNA (Rocchi et al., 2022).

Based on the interaction found between IN and TAR RNA, we speculate that the binding of TAR to IN after the integration step may destabilize and dissociate the integration complex from the nucleosomal site. To investigate this hypothesis, we performed in vitro dissociation experiments of integration complexes reconstituted onto human nucleosomes using various RNA or DNA structures. RNA fragments were found to dissociate integration complexes, whereas DNA structures appear to be much less efficient. This dissociation was confirmed using Biolayer interferometry, on top of showing that TAR RNA element would be the most effective for dissociating integration complex. Furthermore, biochemical characterization of the composition of nucleoprotein complexes released after RNA-induced dissociation revealed that IN.LEDGF/p75 complex was specifically dissociated from the nucleosomal integrated product.

Taken together, our data suggest a new functional interplay between HIV-1 integration and transcription, which needs to be precisely coordinated to allow optimal invasion of chromatin by the virus.

P3* - Towards a molecular understanding of the role of TMEM70 in the biogenesis of the proton channel of F1FO-ATP synthase

Narda Ardda | *IBGC, U5095*

The F1Fo-ATP synthase is a 600 KDa multi-subunit protein complex functioning as a rotating nano-motor that couples the translocation of protons across the inner mitochondrial membrane to the generation of ATP from ADP and Pi in the matrix. The assembly of this enzyme is a very complex and relatively poorly understood process which requires assembly factors; i.e. proteins which are not part of the final complex but which have a critical role in the assembly steps. TMEM70 is one of these assembly factors, located in the inner mitochondrial membrane, which mutations are the most common causes of nuclear ATP synthase deficiencies associated with autosomal recessive neonatal mitochondrial encephalo-cardiomyopathy.

Several research teams, including ours, have shown that TMEM70 is required for the assembly of the subunit-c of the ATP synthase, which is a hydrophobic protein present as 8 identical copies forming a transmembrane oligomeric ring within the ATP synthase proton translocating domain (Fo) [1–3]. Yet the molecular mechanism of TMEM70 is still poorly understood. In order to gain more insights into its structure-function relationships, we decided to study a few selected missense pathogenic mutations of TMEM70. We expressed mutant TMEM70 in our knock-out cell line and started to characterize the impact of these mutations on TMEM70 protein itself, on the ATP synthase levels (and in particular on subunit c), and on the OXPHOS system. Our preliminary results, shown on this poster, reveal differential impacts of the mutations on the TMEM70 structure and function.

1. Carroll, J., He, J., Ding, S., Fearnley, I. M. & Walker, J. E. TMEM70 and TMEM242 help to assemble the rotor ring of human ATP synthase and interact with assembly factors for complex I. Proc. Natl. Acad. Sci. 118, e2100558118 (2021).

2. *Bahri, H. et al. TMEM70 forms oligomeric scaffolds within mitochondrial cristae promoting in situ assembly of mammalian ATP synthase proton channel. Biochim. Biophys. Acta BBA - Mol. Cell Res. 1868, 118942 (2021).*

3. *Kovalčíkova, J. et al. TMEM70 facilitates biogenesis of mammalian ATP synthase by promoting subunit c incorporation into the rotor structure of the enzyme. FASEB J. 33, 14103–14117 (2019).*

P32* - Mechanisms of hypertension lesions: microvascular damage and role of inflammation

Arthur Orieux | BMC, U1034

Hypertension (HTN) affects 1.5 billion people worldwide and is responsible for 8.5 million deaths each year. HTN affects large and small vessels in numerous organs, leading to microvascular damage in the brain (strokes, dementia), eyes (retina), heart (hypertrophic cardiomyopathy), blood vessels (arterial stiffness), and kidneys (chronic kidney disease).

The objective is to study the mechanisms that induce the HTN lesions.

Three-month-old male mice from the 129S1/SvImJ (129/Sv) and C57BL/6J strains were treated with angiotensin II (AngII; 600 ng/kg/min) or 0.9% saline for four weeks. We systematically evaluated markers of vascular injury in the brain, heart, kidneys, and eyes. Both strains developed similar moderate HTN levels induced by AngII. However, only the 129/Sv mice exhibited deficits in spatial learning and memory (Morris Water Maze and Probe test), blood-brain barrier hyperpermeability (dextran 3 kDa), astrocyte activation (GFAP immunostaining), retinal damage, early hypertrophic cardiomyopathy (echocardiography), and renal podocyte lesions (electron microscopy) with albuminuria after AngII.

We performed bulk-RNA sequencing of brain microvessels to identify pathways involved in microvascular damage. In 129/Sv mice treated with AngII, we observed down-regulation of specific inflammatory pathways compared to the C57BL/6J strain, potentially contributing to their vulnerability to HTN-induced damage. These results were supported by increased macrophage recruitment in the hippocampus (IBA1 and CD206 immunostaining), heart and kidney (complement pathway) in C57BL/6J mice after AngII treatment compared to the hypertensive 129/Sv strain. We induced low-grade inflammation via intraperitoneal lipopolysaccharide (LPS) injections (0.25 mg/kg/week) in 129/Sv mice with AngII administration without change in blood pressure. The hypertensive 129/Sv mice with LPS did not develop hypertrophic cardiomyopathy and had significantly lower albuminuria levels compared to the same hypertensive strain without LPS.

These results suggest low-grade systemic inflammation could protect hypertensive microvascular lesions in 129/Sv mice. These results offer promising perspectives for refining inflammation pathways in HTN (type I IFN signaling).

P44* - A new chemokine identified as a key player of tumor immune microenvironment remodeling in B-catenin mutated hepatocellular carcinoma»

Justine Vaché | BRIC, U1312

Patented work

The emergence of immunotherapy for the treatment of advanced hepatocellular carcinoma (HCC) has significantly improved overall patient survival. However, accumulating evidence has demonstrated that 2/3 of HCC harboring oncogenic mutations of the B-catenin coding gene (CTNNB1) are devoid of immune infiltrates and resistant to immunotherapy. To further elucidate this immune escape process, we developed two 3D liver cancer spheroid models using a B-catenin-wild-type cell line (Huh7) and a B-catenin-mutated cell line (HepG2). We then evaluated immune cell infiltration by flow cytometry and demonstrated that mutated B-catenin significantly reduces the infiltration of immune cells, particularly Natural Killer cells. This localized immunosuppression mediated by mutated B-catenin could result from a deregulated production of chemokines, which are key player molecules involved in cancer cells-immune cells communication. Our global transcriptomic analysis carried out in HepG2 cell model revealed a deregulation of a particular chemokine, referred to here as CXX, mediated by mutated-B-catenin. At the protein level, depletion of the mutated form of B-catenin induced an increase in CXX expression and secretion in both 2D and 3D models. On the contrary, we observed reduced expression and secretion of this chemokine following B-catenin pathway overactivation in the Huh7 models. Consistent with these results, in silico transcriptomic analysis in a cohort of HCC patients revealed that mutated B-catenin represses CXX expression. Moreover, we report both a decrease in its expression and a reduced infiltration of NK cells in tumor sections from HCC patients with B-catenin mutation. As CXX is known to recruit NK cells, our future experiments will focus on analyzing the impact of this chemokine on their recruitment in our liver tumor spheroid models.

In conclusion, our results identify CXX as a novel negative target of B-catenin and provide new insights into the functioning of HCC tumor microenvironment in a context of B-catenin mutation.

Hepatocellular carcinoma, B-catenin, chemokines, immune escape, immunotherapy

First scientific session

Chairs: Lucie Brisson & Mathieu Metifiot

Margaux Laisné | BMC, U1034

«Astrocytic serglycin exhibits ambivalent properties in the control of astrogliosis in the pathophysiology of multiple sclerosis»

Introduction : During neuroinflammation, such as multiple sclerosis (MS), astrocytes become reactive, undergoing morphological and molecular changes named «astrogliosis». This phenomenon can be beneficial, with the establishment of a glial scar limiting the size of inflammatory lesions, or deleterious, with the production of pro-inflammatory factors leading to blood-brain barrier (BBB) opening. To characterize astrocytes signature during astrogliosis, we performed an RNA sequencing on "basal" (hBA) versus "reactive" human astrocytes (hRA) and identified Serglycin (SRGN), a proteoglycan, as upregulated by hRA.

Aim : The aim of our project is to elucidate the role of astrocytic SRGN and its players during neuroinflammation.

Methods : In vitro, we used hRA transfected with SRGN siRNAs and Human Brain Microvascular Endothelial Cells (hBMECs) incubated with hRA conditioned medium to perform transcriptomic and proteomic analysis. In vivo, we used inducible astrocytic-specific SRGN knockout mice (SrgnACKO) and induced acute inflammatory lesion via intracortical stereotactic injection or experimental autoimmune encephalomyelitis (EAE), a mice model of MS.

Results: In vitro, in a model of astrogliosis, we demonstrated that astrocytic SRGN overexpression contributes to astrocytes hyperplasia, elongation and the expression of astrocytic tight junctions. In vivo, glial scar formation is delayed in SrgnACKO compared to control mice correlated with less astrocytic reactivity (GFAP, VIM). Conversely, we showed, in vitro, that astrocytic SRGN promotes a chemokine-rich (CCL5, CCL7) pro-inflammatory secretum that impairs the BBB integrity, since it alters endothelial junction gene expression (OCLN, CADH5, ZO-1). Interestingly, preliminary results of EAE show that SrgnACKO mice exhibit less severe disability score.

Discussion: Collectively, our data suggest that, in acute and chronic neuroinflammatory contexts, like in MS, SRGN dichotomous role on astrogliosis may favor the establishment of a protective glial scar, on one hand, and promote a pro-inflammatory secretum deleterious for the BBB homeostasis, on the other hand.

Arthur Poulet | BRIC, U1312

«Exploring Nat8L involvement in HSC self-renewal/expansion processes upon stress»

Hematopoietic homeostasis within the blood compartment is maintained in the bone marrow (BM) by a rare population of hematopoietic stem cells (HSCs). These HSCs can self-renew, differentiate, and expand to meet the organism's needs. During embryonic development, HSCs emerge from the fetal liver, undergo an expansion phase, and subsequently migrate to the BM. Metabolomic analysis conducted by our laboratory revealed that N-Acetyl-L-Aspartate (NAA) levels in expanding fetal liver HSCs are up to ten times higher than in quiescent BM HSCs. This suggests a potential role for NAA in HSC expansion. Although NAA is abundant in the brain, its role in hematopoiesis remains unexplored. To investigate NAA's function in HSC expansion, we are studying physiological conditions inducing HSC expansion, such as hematopoietic stress following infections or myeloablative treatments.

To study NAA's role in hematopoiesis, we utilized NAT8L gene-deficient transgenic mice (Nat8l-KO), lacking the enzyme responsible for NAA synthesis. Functional and phenotypic analyses were performed after serial injections of lipopolysaccharides (LPS) or 5-FU to induce hematopoietic stress in Nat8l-KO mice.

Under basal conditions, no significant differences were observed between Nat8l-KO and control mice in HSC. However, following 5-FU treatment, Nat8l-KO mice exhibited reduced survival rates, frequency, and numbers of HSCs in the BM, confirming NAA's critical role under hematopoietic stress. To investigate NAA's early response in HSCs to stress, we analyzed NAT8L expression levels and conducted kinetic assessments of NAA production in HSCs after LPS exposure. Initial findings indicate a rapid decrease in Nat8l levels shortly after stress exposure. Ongoing analysis aims to elucidate subsequent changes in NAA metabolite levels during hematopoietic regeneration. Future transplantation experiments will assess the functional capabilities of stress-exposed HSCs from Nat8l-KO mice. Altogether, these results highlight the critical role of NAA in stress hematopoiesis and expansion of HSCs, providing new insights into hematopoietic adaptation under challenging conditions.

Elina Mercier | MRGM, U1211

«OCA2 exon 10 splicing: of mice and men»

Twenty genes have been identified that are associated to albinism in human. Among these, OCA2 encoding the melanosomal transporter P protein is of particular interest. Pathogenic variants in this gene solve 30% of genetic diagnosis. The major transcript of OCA2 carries all 24 exons. Noteworthy, a minor transcript that lacks the in-frame exon 10, is expressed in healthy individuals, but to date, the corresponding putative protein remains undescribed. In a previous study, we showed that some rare SNP including synonymous variants of exon 10, significantly increase skipping resulting in albinism, presumably by lack of functional protein production (Michaud et al., PCMR 2023). Recently, we screened unsolved OCA2 patients and identified supplementary variants distributed all along exon 10 that also induce skipping.

This study shows that exon 10 of OCA2 is highly sensitive to skipping and suggests that there is a quantitative threshold beyond which albinism will manifest itself. This hypothesis leads us to investigate critical sequences that control the retention/skip balance. We combine three approaches: 1- correlation analysis in healthy individuals (benign SNPs/ pigmentation index/

exon 10 skip); 2- functional studies based on non-human mammalian sequences. For instance, the mouse OCA2 exon 10, which is highly homologous to the human sequence, is fully retained after splicing. Combination of murine and human sequences in a minigene assay indicate that exon 10 skipping relies on a combination of sequences in the exon, and in both upstream and downstream introns. 3- identification of the splicing mechanisms and regulation using antisens oligonucleotide and RNA pull-down assay.

All in all, this study improves OCA2 diagnosis and should contribute to increased efficiency of in-silico prediction of pathogenic variants. More, we should gain insight into the way OCA2, a major pigmentation regulator has evolved not only in different human populations but also in separate mammalian species.

Aurélien Richard | TBMCore

«3D in vitro models of the normal human breast and breast cancer»

The LP2N/BiOf (UMR5298) develops a new co-extrusion microfluidic technique, called the Cellular Capsule Technology (CCT), designed to produce 3D encapsulated organoids and tumoroids in spherical or tubular alginate capsules. This technique, which has been patented several times and transferred to the VoxCell facility (UAR TBMCore in Bordeaux) is used for developing innovative 3D in vitro models that better recapitulate real tissues compared to flat 2D culture.

In the context of oncology research, these new models are much sought after because of their relevance for 1/ the study of the physiopathology and the microenvironment of various cell types, and 2/ the study of therapeutic molecule diffusion in a 3D tissue. Thanks to its biocompatibility, high porosity and rheological properties, alginate allows 3D self-organization and proliferation of cells in a confined or semi-confined environment where every parameter is tunable: cell type/density (mono/coculture), capsule rigidity and matrix composition and concentration.

Thanks to the CCT, I was able to develop new 3D in vitro models of the mammary epithelium and stroma. The first model consists of an approximately 550 µm hollow tubular capsule with a central lumen formed by a mammary epithelium made of MCF10A cells, attached onto the alginate inner wall via a layer of Matrigel. The second and third models consist of the same tubular capsule but with either MCF7 or MDA-MB-231 cancer cells. The fourth model consists of the same capsule with a mammary epithelium (MCF10A) attached onto a second layer of tissue made of fibroblasts that are embedded in an alginate-collagen I-Matrigel composite hydrogel, in order to reproduce the complexity of the surrounding tissue.

In the context of normal breast and breast cancer research, it has been observed that the microenvironment plays a major role in cell behavior. Our main goals are 1/ to develop different models of the normal breast and breast cancer with different levels of complexity to study specific aspects of the mammary tissue and 2/ to compare our different models to understand how the changes in the microenvironment (cell types, matrices...) can affect cell behavior.

Chloé Lambert | MFP, U5234

«BILBO1, BILBO2 and companions: more than a trilogy?»

Trypanosoma brucei brucei (T. brucei) is a unicellular, and flagellated parasite responsible for human and animal African Trypanosomiasis. Its flagellum exits the cell body through the flagellar pocket (FP). The FP is formed by an invagination of the plasma membrane at the base of the flagellum and is the unique site of endo and exocytosis. The FP is maintained enclosed around the flagellum by the flagellar pocket collar (FPC), an essential but poorly understood cytoskeletal structure in terms of its composition, structure and function.

Our team previously identified and characterized two key FPC proteins, BILBO1 and BILBO2. Their N-terminals domains (NTD) share a structural similarity, with several conserved essential residues. Moreover, they have demonstrated that another FPC protein, FPC4, interacts with the NTD of BILBO1 and BILBO2.

We have now identified three other proteins whose NTDs share 30% homology with the BILBO1-NTD, forming a novel trypanosome-specific protein family. Among these proteins is BILBO3 an uncharacterized protein. Preliminary in silico and in vitro data suggest a similar role and function for these NTD domains. The aim of this work is to characterize BILBO3 by studying its localization, function and interactome.

I have shown that BILBO3 localizes at different cytoskeletal structures, suggesting it may play critical roles in the biogenesis of the FPC. Further using BioID, I identified several previously uncharacterized BILBO3 interaction partners, revealing a higher level of complexity in the components involved in FPC structure and function than initially expected.

Second keynote speaker

Chairs: Jan Pieter Konsman & Manuel Rojo

Stephan Güttinger | University of Exeter, UK

«Replicability is not a good standard for research quality»

Replicability is often seen as a key indicator for the quality of research: good scientific work can be replicated by other researchers. If too many replications in a discipline fail, then this indicates that the research in this field is no longer of the required quality. The source of the problem is usually traced back to the behaviour of researchers, in particular their use of so-called "questionable research practices" (QRP). These practices are used either because the researchers lack proper methodological training or because they have fallen prey to the pressures of the "publish-or-perish" culture in science. The result in both cases is "sloppy" science.

In my talk I will take a critical look at this way of thinking about replicability. I will first reflect on the wide-ranging consequences that the recent worry about a "crisis" of replicability has for science. In particular, I will highlight how talk of a replication crisis has been used a) to intensify the displacement of human researchers from the experimental process (via automation) and b) to demonise research practices that are not necessarily problematic. I will argue that these developments are problematic for science and, more importantly, unnecessary because replicability is not, and should not be, the gold standard for research quality.

Biography:

Stephan Güttinger is a lecturer in Philosophy of Data and Data Ethics at the University of Exeter, UK. He is co-lead of the Data, Knowledge and AI research strand at the Egenis Centre (University of Exeter). He is also the academic lead for Exeter of the Ethical Data Initiative, which is jointly hosted by the University of Exeter and the Technische Universität München (TUM). Stephan's work focuses on knowledge production in the biological laboratory, with a particular focus on issues such as experimental control, reproducibility, and the increasing use of robot science (or "machine learning-enabled automation"). He also holds a season ticket for Tottenham Hotspur and thinks Arsenal should be re-named Woolwich FC.

Second scientific session

Chairs: Jan Pieter Konsman & Manuel Rojo

Delphine Fessart | BRIC, U1312

«Gynaecological Cancers: Ageing, Proteostasis Dysfunction, and Resistance Mechanisms»

Gynaecological cancers predominantly affect older women and are characterised by significant genomic instability. This instability, a hallmark shared by both ageing and cancer, induces endoplasmic reticulum (ER) stress, driving cellular reprogramming and facilitating cancer cell adaptation. Ageing is a critical risk factor for cancer development, with proteostasis dysfunction—a hallmark of many age-related diseases—playing a pivotal role. Proteostasis, or protein homeostasis, ensures proper protein folding, conformation, and degradation through a network of molecular chaperones and proteolytic machinery. Over time, the capacity of this network declines due to accumulated endogenous and external stressors. This leads to protein aggregation and compromised proteome integrity. Senescence, a cellular stress response characterised by irreversible growth arrest under conditions such as oncogene activation, acts as a key barrier to early tumour formation. For instance, oncogene-induced senescence (OIS), triggered by the activation of oncogenes like RAS, functions as a critical tumour-suppressive mechanism. Overcoming this barrier is essential for tumour initiation and progression.

Our team has identified a pivotal role for the chaperone protein AGR2, located in the ER and secreted extracellularly as eAGR2, in regulating proteostasis and cancer development. Our findings demonstrate that AGR2 contributes to bypassing the barrier posed by OIS, thereby enabling tumour initiation. This positions AGR2 as a potential anti-senescence protein, driving oncogenesis by promoting cellular adaptation. In addition, we have uncovered a role for AGR2 in resistance to chemotherapy-induced senescence. Our data suggest that targeting AGR2 could restore chemotherapy-induced senescence and improve treatment sensitivity. Our research aims to elucidate the mechanisms by which proteostasis dysfunction enables cells to overcome senescence, promotes tumour initiation, and drives chemoresistance in gynaecological cancers.

Adrien Birot | IBGC, U5095

«Unraveling the regulation of cohesin through phosphorylation»

Cohesin is a multiprotein complex that mediates the capture of DNA molecules. Trans DNA capture enables sister chromatid cohesion, critical for DNA double-strand break repair and accurate chromosome segregation during nuclear divisions. Conversely, cis DNA capture facilitates the formation of chromatin loops, which define the functional three-dimensional architecture of genomes during interphase. Given its broad functional roles, we aim to investigate how cohesin functions are regulated spatially and temporally.

Our recent forward genetic screens in Schizosaccharomyces pombe have identified kinases as critical regulators of cohesin. The CDK5 orthologue Pef1 and TORC1 kinases modulate phosphorylation of key residues on cohesin subunits, influencing its chromatin interaction and chromosome segregation in sister chromatid cohesion mutants.

To further explore the regulatory potential of phosphorylation, we are conducting a large-scale reverse genetics screen, mutating each phosphorylated residue to a non-phosphorylatable (alanine) or phosphomimetic (glutamate/aspartate) state. Ongoing phenomic assays suggest the identification of novel regulatory pathways, particularly in cohesin's role in cellular adaptation to environmental changes, highlighting its dynamic regulatory capacity.

These findings offer new insights into the phosphorylation-mediated control of cohesin and its broader impact on cellular processes.

Third scientific session

Chairs: Marija Vlaski-Lafarge & Vanja Sisirak

Audrey Burban | IBGC, U5095

«Targeting NLE1 signaling in glioblastoma»

Brain tumour stem cells (BTSCs) are a population of self-renewing malignant stem cells that play an important role in glioblastoma tumour hierarchy and contribute to tumour growth, therapeutic resistance, and tumour relapse. Thus, targeting of BTSCs within the bulk of tumours represents a crucial therapeutic strategy. Here, we report that Edaravone is a potent drug that impairs BTSCs in glioblastoma. We show that Edaravone inhibits the self-renewal and growth of BTSCs harbouring a diverse range of oncogenic mutations without affecting non-oncogenic neural stem cells. Global gene expression analysis revealed that Edaravone significantly alters BTSC transcriptome and attenuates the expression of a large panel of genes involved in cell cycle progression, stemness, and DNA repair mechanisms. Mechanistically, we discovered that Edaravone directly targets Notchless homolog 1 (NLE1) and impairs Notch signalling pathway, alters the expression of stem cell markers, and sensitizes BTSC response to ionizing radiation (IR)-induced cell death. Importantly, we show that Edaravone treatment in preclinical models delays glioblastoma tumourigenesis, sensitizes their response to IR and prolongs the lifespan of animals. Our data suggest that repurposing of Edaravone is a promising therapeutic strategy for glioblastoma patients.

Asen Daskalov | *Immunoconcept, U5164*

«Regulated cell death in fungi from a comparative immunology perspective»

The death of fungal cells has been studied in a variety of contexts including responses to antifungal drugs, during fungal developmental processes, in response to bacterial or mycoviral fungal pathogens, and during non-self-recognition between distinct strains of the same species (allorecognition). Some of the genetic determinants and molecular mechanisms of fungal cell death processes are beginning to be understood in detail. Recent advancements have uncovered fungal cell death machinery that shares ancestry with key actors of immune cell death in other eukaryotic and prokaryotic taxa. Transkingdom evolutionary links include fungal molecular sensors such as the NOD-like receptors and signaling domains related to the TIR (Toll/interleukin-1 receptor) family, which are a staple of immunity throughout the tree of life. Downstream cell death executioner proteins homologous to pore-forming proteins that mediate mammalian necroptosis and pyroptosis are also abundant and widespread in fungi, especially in Ascomycota. These findings prompt us to speculate on the possible origins of fungal cell death and reconsider fungal innate immunity beyond allorecognition.

Eloise Bertiaux | *MFP, U5234*

«Deciphering the role of the luminal distal proteins in the centriole elongation and architecture»

The centriole, a cylindrical organelle composed of nine microtubule triplets, is a key component of most eukaryotic cells. Found in pairs surrounded by pericentriolar material, centrioles collectively form the centrosome, the primary microtubule-organizing center of the cell. Additionally, centrioles can dock at the plasma membrane to nucleate the formation of cilia on the cell surface.

Recent advancements in cryo-electron microscopy (cryo-EM) combined with ultrastructure expansion microscopy (U-ExM) have enabled detailed characterization of centriole structure and protein composition. Studies revealed that the central region of the centriole is enveloped by a dense helical structure comprising at least four distinct proteins. Similar structural and compositional insights were obtained for the proximal region, where the cryo-EM-described cartwheel structure—a critical feature for centriole duplication—aligns precisely with protein mapping achieved using U-ExM.

However, the inner distal region of the centriole remains poorly understood. Specifically, it is unclear whether this region is highly organized or composed of amorphous material. In this study, we identified an inner ring structure at the distal end of the centriole, comprising two proteins, C2CD3 and NA14. This ring appears to play a critical role in regulating centriole length and the positioning of distal appendages. We propose that this conserved structure serves as a crucial link between the inner part and the outer components of the centriole, shedding light on its architectural and functional integration.

Poster session

***Poster in competition**

P1* - Deciphering Sézary Syndrome Tumoral Heterogeneity Through Bioinformatic Approach SOBIO

Nasir Mahmood Abbasi | *BRIC, U1312*

Sézary syndrome (SS) is a rare and aggressive cutaneous T-cell lymphoma characterized by erythroderma, lymphadenopathy, and circulating malignant CD4+ T-cells. The cellular origins and differentiation stage of SS remain unclear, with recent studies suggesting significant inter- and intra-heterogeneity.
This study aims to explore the signature and heterogeneity of Sézary syndrome using advanced bioinformatics techniques applied to single-cell RNA sequencing (scRNA-Seq) data.
Our team developed seven Sézary cell lines derived from three different patients; L1, L2 are derived from Patient 1 (P1), L3 and L4 derived from Patient 2 (P2) and L5, L6 and L7 cell lines derived from Patient 3 (P3). The methodology involves scRNA-Seq, CITE-Seq, and TCR sequencing using the 10X Genomics platform.
Our results reveal significant diversity among SS cell lines, with patient-dependent clustering observed through UMAP visualization. Eighteen distinct clusters were identified, each exhibiting unique transcriptomic profiles. The analysis was structured along three axes: (1) comparing all patient-derived cell lines to healthy CD4+ T-cells, (2) investigating inter-heterogeneity between and within patient cell lines, and (3) studying intra-heterogeneity within individual cell lines. Key findings include the upregulation of known SS signature markers like KIR3DL2 and TWIST1, as well as potential novel markers such as BCAT1 and EPCAM. Patient-derived cell lines gene expression patterns and pathway enrichments were observed, supporting the concept of inter-heterogeneity. Notably, cell lines derived from the same patient showed differentially expressed genes and upregulated pathways, indicating transcriptomic differences despite shared origin.
The study confirms the presence of significant inter-heterogeneity at both patient and cell line levels. However, the assessment of intra-heterogeneity within individual cell lines was less conclusive, with minimal gene expression differences observed between clusters. This aspect requires further investigation, possibly involving refinement of filtering parameters. This comprehensive analysis provides valuable insights into the molecular landscape of Sézary syndrome, highlighting its complex and heterogeneous nature. The identification of unique gene expression signatures and potential novel markers could have implications for improved diagnosis and targeted therapies. Furthermore, the observed heterogeneity underscores the importance of personalised approaches in managing this aggressive malignancy.
Future research directions may include functional studies to validate the role of newly identified markers, exploration of the minimal intra-heterogeneity observed within cell lines, and investigation of the potential clinical implications of the heterogeneity patterns. This work contributes significantly to our understanding of Sézary syndrome and may pave the way for more effective, personalised treatment strategies in the future.

Keywords: Sézary syndrome, Cutaneous T-cell lymphoma, Single-cell RNA sequencing (scRNA-Seq), CITE-Seq, TCR sequencing, 10X Genomics

P2* - Study of myeloid tumor microenvironment in cutaneous T-cell lymphomas

Amandine Roussel| *BRIC, U1312*

Cutaneous T-cell lymphomas (CTCL) correspond to an abnormal accumulation of T-cells primarily in the skin and represent 70% of cutaneous lymphomas composed of entities with variable prognosis. Mycosis Fungoides are indolent for several years, whereas Sézary Syndrome are more aggressive with development of tumors in the skin and blood involvement (Werner Kempf, Hematol Oncol, 2021;PMID: 34105822). With the exception of hematopoietic stem cell transplantation (only for <65 years old patients), there are no effective or sustainable therapies for advanced CTCL (De Masson et al, Lancet, 2023; PMID: 37105210) even if the development of targeted immunotherapies has improved the prognosis of patients (Bozonnat et al, EClinicalMedicine, 2024;PMID: 39007062). This lack of efficient therapies could be due to immune state of the tumor microenvironment (TME) (Phillips et al, Nature communications, 2021;PMID: 34795254).

In this context, our team, focusing on TME, has demonstrated a huge CD163+ macrophages infiltration in patient skin biopsies. The aim of our project is to better understand the dialog between tumor-associated macrophages (TAMs) and CTCL.

We have co-cultivated in 2D culture healthy CD14+ monocytes/macrophages with CTCL cells derived from patient samples (Oncodermatology unit, Bordeaux University hospital) to evaluate their influence on each-other. Using flow cytometry, we observed that macrophages increase PD-L1 expression when they are in direct or indirect contact with tumors cells. This is associated with an increased immunosuppression activity of the “educated” monocytes/macrophages on CD4+ T lymphocytes from healthy donors, that may contribute to tumor escape. The next steps will be to study the secretome (proteome profiler, LegendPlex) or molecular mechanisms (RNAseq) involved in such effects. This will help us to identify new targets which we plan to validate using a 3D spheroid culture models including extracellular matrix, already developed in the lab, (Lamaison C, Blood Advances, 2021;PMID: 34555842).

This work will allow us characterize the role of these TAMs on CTCL properties and vice versa, to better understand the impact of TME on tumor survival and escape, and develop alternative therapies.

P4* - Characterization of a protein involved in lipid droplet biology in *Trypanosoma brucei*

Kathyanna Arnould | *iMET*

This project aims to advance the understanding of proteins involved in lipid droplet biology in trypanosomes. Lipid droplets are conserved organelles across eukaryotes and play a critical role in lipid storage. Their formation is regulated by the Seipin protein. The objective of this study is to determine if the candidate gene Tb1125.4.3820 encodes the Seipin protein in *T. brucei*. A functional genomics approach was employed, including endogenous tagging for protein localization and a knock-out strategy to determine its function, utilizing CRISPR/Cas9 genome-editing technology. The results indicate that the protein localizes to the endoplasmic reticulum. Its absence leads to heterogeneity in lipid droplet size and also impacts their number, suggesting a role in lipid droplet formation and regulation. These findings are consistent with previous studies on Seipin in other organisms, such as Homo sapiens and *Saccharomyces cerevisiae*.

P5 - TileScooe: Integrating tessellation-based quantification with spatial omics for improved gene colocalization analysis of the tumor microenvironment

Maialen Arrieta Lobo | *IBGC, U5095*

Over the past few years, numerous algorithms and computational tools have been developed to address the complex issue of spatial colocalization in spatial transcriptomics samples. However, current methods1 lack computational robustness in quantifying different patterns of spatial correlation between genes on 10X Visium samples and do not offer precise statistics on the spatial distribution of such pairs of correlated genes. To enhance the accuracy of describing spatial colocalization of genes, we developed an approach that integrates techniques from various disciplines, including spatial omics and geospatial science. In this presentation we will discuss our method as well as illustrate it with examples of spatial colocalization analysis of glioblastoma Visium data.

Tessellation-based expression quantification has been utilized in fields such as single molecule localization microscopy. Here we introduce this technique, coupled with previous work on spatial measurements of molecules on a subcellular level (DypFISH2), as a foundation for analyzing patterns in the spatial distribution of expression within spatial transcriptomic datasets. Our process begins with spot clustering followed by random tessellation of spots, per cluster. We then retrieve the expression of the two genes of interest in each tessellation tile, converting this information into density vectors. These vectors are subsequently analyzed using several spatially-aware metrics, yielding a statistically robust quantification of gene colocalization.

Applying our method to human (Ravi et al. 2022) and our own patient-derived xenograft GBM 10X Visium slides addresses the unique challenge of identifying and quantifying gene spatial colocalization within the tumor microenvironment of glioblastoma patients biopsies in order to tackle the mechanisms driving tumor progression. Indeed, this environment's complexity, characterized by a diverse cellular composition and dynamic interactions among cancer cells, immune cells, and stromal elements, makes it crucial to understand the spatial organization of genes.

P6* - Rôle complexe des récepteurs Toll-like dans la physiopathologie du lupus

Marine Aupetit | *ImmunoConcEpT, U5164*

Le Lupus Érythémateux Systémique (LES) est une maladie auto-immune chronique, actuellement traitée par immunosuppresseurs, induisant des effets secondaires. Identifier les mécanismes responsables de l'activation des lymphocytes autoréactifs permettrait de développer des thérapies ciblées. Les lymphocytes B (LB) autoréactifs jouent un rôle central dans la physiopathologie du lupus, parce qu'ils produisent des autoanticorps et activent des lymphocytes T autoréactifs qui infiltrent les organes, et provoquant ainsi leur dysfonction. Ces LB sont activés par les Toll-like receptors 7 et 9 (TLR), qui vont reconnaître une accumulation anormale d'autoantigènes (ARN et ADN). De façon inattendue, TLR7 et 9 ont des rôles opposés sur la sévérité du lupus. TLR7 est pathogène alors que TLR9 est protecteur. Étudier pourquoi ces deux récepteurs, supposés homologues, n'ont pas la même fonction est l'objet de ce projet. Nous émettons l'hypothèse que les domaines de signalisation TIR de TLR7 et TLR9 engendrent des cascades de signalisation différentes. Pour la tester, nous avons créé un TLR9 où seul le domaine TIR a été remplacé par celui du TLR7 (TLR9TIR7). Ce TLR9 modifié a été introduit dans le locus endogène de TLR9 par CRISPR Cas9 dans un modèle de souris lupique MRL/lpr.

Ce modèle nous permet d'étudier in vitro la signalisation des LB spléniques porteurs soit de TLR9TIR7, soit d'un TLR9 sauvage (TLR9WT) stimulés avec le même agoniste, le CpG (agoniste du TLR9). Nous caractérisons les voies de signalisation induites par le CpG dans des LB TLR9WT par Western Blot. Le CpG induit la phosphorylation de P-65 (voie MyD88/NF-κB) et IRF7, qui sont maximales après une heure de stimulation. Nous comparerons ensuite si la nature, la cinétique ou l'intensité d'activation des voies de signalisation sont différentes dans les LB TLR9TIR7 versus TLR9WT. Identifier ces différences permettrait de développer de nouvelles stratégies pour stimuler la voie protectrice de TLR9 ou inhiber celle pathogène de TLR7.

P9* - A cellular assay to determine the fusion capacity and pathogenecity of MFN2 variants linked to Charcot-Marie-Tooth disease of type 2A

Chloé Barsa | *IBGC, U5095*

Charcot-Marie-Tooth Disease is the most common inherited neuromuscular disease with a prevalence of 1 in 3,300 individuals worldwide. The most common causes of CMT2A, a subtype of the inherited disease characterized by muscle weakness and loss of sensation in the body's extremities, are mutations in the mitofusin2 gene. Mitofusin2 (Mfn2) is an essential mitochondrial dynamin-related GTPase and is responsible for the outer mitochondrial membrane fusion. Besides its necessity for the morphological integrity of the respiratory organelle, Mfn2 also plays a role in mitochondrial mobility and mitochondria-endoplasmic reticulum juxtaposition, and allows the maintenance of mitochondrial DNA content and oxidative phosphorylation. From the literature, we know that some mitofusin2 variants retain a majorly filamentous mitochondrial network while still being pathogenic Therefore, what are the mechanisms that may be responsible for the pathogenicity of fusion-competent CMT2A variants?

In this study, we seek to characterize the functional consequences of pathogenic Mfn2 mutations and the properties of Mfn2 variants in order to understand the different processes governed by Mfn2 and which could potentially allow us to provide a functional diagnosis to CMT2A patients.

P10* - Circularization, transcription and transfer capacity of integrative and conjugative elements of *Mycoplasma hominis*, a human genital pathogen

Bachir Boureima Abdou | *MFP, U5234*

Integrative and conjugative elements (ICEs) are modular mobile genetic elements that disseminate through excision, circularization, and transfer. In Mycoplasma hominis, a human urogenital pathogen, 45% of isolates harbor ICEs integrated into the chromosome. These ICEs, measuring 27-30 kbp, contain 25-30 CDSs. This study aimed to assess ICE circularization capacities under various environmental conditions and investigate the transcription of ICE CDSs. The second part focused on the transfer capacities of M. hominis ICE through mating experiments.

Methods
Using qPCR and RT-qPCR, we assessed the circularization and transcription of the M. hominis 4788 strain ICE (ICEHo-4788) under conditions including exponential/stationary growth phases, mitomycin C exposure, cold/hot shock stress, and growth in HeLa cell culture.

Functional studies involved mating experiments between donor strains carrying one ICE and recipient strains in both axenic and cell culture conditions.

Results and Conclusion
In axenic growth, a circularization peak occurred at 12 hours of culture, with a 9.4-fold increase in circular forms compared to the reference condition. Exposure to mitomycin C and cold-shock stress resulted in 3.3- and 3.0-fold rises in circular forms, respectively. Maximum circularization in cell culture was observed at 72 hours and 7 days post-infection, with 10- and 23-fold increases, respectively. RT-qPCR experiments showed that all ICEHo-4788 CDSs were transcribed as a single polycistronic mRNA, with significant transcription increases in cell culture conditions at 7 days post-infection. Although no ICE transfer was achieved, a "Mycoplasma chromosomal transfer" was observed, where the recipient strain transferred chromosome regions to the donor strain, creating mosaic genomes. This study emphasizes the influence of environmental conditions on ICEHo-4788 circularization dynamics and transcription, along with evidence of genetic material exchange in M. hominis.

Keywords: Mycoplasma hominis, ICE, Circularisation, transcription, Mycoplasma chromosomal transfer.

P11* - Src promotes tumor cell invasion by hijacking the translation machinery

Anouk Chatefau | *BRIC, U1312*

The Src oncogene controls cancer cell invasiveness by promoting invadosome formation and extracellular matrix degradation (ECM). Invadosomes are enriched in the eukaryotic translation initiation factor 3 (eIF3) complex associated with a local mRNA translation activity mandatory for their maintenance. Here, we show that Src regulates mRNA translation by controlling the expression of eIF3 subunits. Among them, eIF3h/e/d are essential for invadosome formation and ECM degradation. We demonstrate that Src controls the canonical mTOR/eIF4E and the non-canonical eIF3d cap-dependent translation initiation pathways. We show that both pathways are necessary for invadosome formation and their ECM degradation function. Finally, we highlighted a correlation between Src and eIF3h/e/d overexpression, which is associated with poor prognosis in hepatocellular carcinoma (HCC) patients and controls the ECM degradation and invasive properties of HCC cells. These findings identify Src as a major regulator of translation initiation pathways, which leads to invadosome formation, ECM degradation and tumor cell invasion.

P12* - Telocytes : Are they implicated in Squamous Cell Carcinoma?

Julie Clachet | *BRIC, U1312*

The skin, being the largest organ in humans, is susceptible to various diseases, including cancer. Among these, skin cancer, encompassing carcinomas and melanomas, ranks as one of the most prevalent globally. Preceding the onset of squamous cell carcinoma (cSCC), pre-cancerous lesions may occur, often making accurate prognostication of their progression difficult.
The epidermis, primarily affected by irradiation, undergoes changes that extend into the dermis, where critical cellular components reside. Notably, telocytes (TC), a recently discovered cell type distinct from fibroblasts, undergo modifications concurrent with dermal structural alterations. As lesions evolve into cancer, a cascade of events unfolds, culminating in invasion

into the dermis and remodeling of the extracellular matrix (ECM) through interactions between cancer cells, neighboring cells, and the ECM.
Literature has established the essential role of fibroblasts in cancer progression, while the role of telocytes remains unexplored. This study aims to identify telocytes as potential markers for the fate of pre-cancerous lesions and elucidate the communication networks among cancer cells, telocytes, and the ECM at various stages of skin cancer progression.
The first part of this PhD project will investigate telocyte behavior during carcinoma progression by identifying telocytes using immunohistochemistry (IHC) staining on cSCC samples at different stages. Concurrently, an in vitro study will focus on the behavior of TC. The mutual effects of telocytes and A431 cells (cSCC cell line) will be examined on proliferation and invasion assays in both 2D and 3D models, as part of a comprehensive characterization process of this particular cell type.
In conclusion, this work aims to highlight the role of telocytes in the progression of squamous cell carcinoma and provide new insights into this poorly understood and little-known cell type.

P14* - Quiescent yeast cells properties during prolonged stationary phase

Pauline Foliard | *IBGC, U5095*

Most cells spend part of their life in quiescence, a reversible proliferation arrest. Although poorly understood, quiescence is central to several essential processes, including tissue homeostasis, development, and aging. Theories of aging have assumed that quiescent cells must cope with the accumulation of damaged macromolecules while retaining their ability to reproliferate to ensure cell renewal. Until recently, it was assumed that aging leads to a monotonic decline in cell survival and reproliferative capacity.

However, recent experiments suggest that the aging process is more complex than a simple and inexorable decline to death. Saccharomyces cerevisiae has played a central role in the study of aging by studying the behavior of cells in stationary phase. Among the phenomena that make ageing studies more complex is the central question of the non-growing state, since a population in stationary phase is assumed to be made up of cells that have all stopped growing for the same period of time.

As it has been suggested that in both bacteria and yeast, cell death during stationary phase may release nutrients to induce cell regrowth, we have addressed the question of the non-growing cell state during extended stationary phase.
Our experiments show that such cell regrowth was never observed, even in long-term culture. We are now trying to generalize this approach to other yeast strain backgrounds and media. With the idea that high mortality might trigger regrowth, we artificially induced the death of a quarter of the stationary phase population. Using early, mid and late proliferation reporters, we found no regrowth or fitness advantage to having dead cells in culture. Finally, we studied the process of quiescence deepening, a phenomenon that blur the understanding of aging.

P15* - High-resolution imaging of brain tumor invasion

Emmanuelle Georget | *BRIC, U1312*

Glioblastoma (GBM) is the most aggressive adult brain tumor, characterized by its highly invasive nature and devastating impact on patient quality of life. The mechanisms that underlie GBM's ability to infiltrate healthy brain tissue (parenchyma) along diverse trajectories, including blood vessels, white matter tracts, and the leptomeningeal space are still poorly

understood. Recent advances in high-resolution tissue imaging techniques offer new possibilities to dissect these invasion mechanisms.

We have developed an experimental pipeline to investigate the interactions between GBM cells and their brain tissue environment, focusing on the migratory pathways of invading tumor cells. To this end, we used two distinct glioblastoma animal models: a syngenic model using adherent mouse cell line (CT2A) injected into C57BL6/N mice, and a xenogenic model using human GBM cells (BTSC73) injected into immuno-deficient (RAG1) mice. In both cases, the tumor cells were fluorescently labeled with YFP.

We used 2-photon shadow imaging (TUSHI) in somatosensory and motor cortex in vivo and in acute brain slices to track the invading tumor cells and to visualize the anatomical tissue context at different stages of tumor progression. Notably, perivascular spaces of capillaries were larger in RAG1 mice injected with GBM cells than in control mice, while capillary diameters did not differ.

To track tumor progression on a macroscale level we implemented a brain clearing protocol (Adipoclear+) and used light-sheet microscopy to visualize proliferation and migration of GBM cells in the entire brain.

Overall, this project leverages cutting-edge microscopy techniques to elucidate the complex mechanisms of GBM invasion, paving the way for the development of novel therapeutic strategies to combat brain cancer.

P16* - Endoplasmic reticulum proteostasis in High grade serous ovarian cancer

Marianne Guilbard | *BRIC, U1312*

Gynaecological malignancies remain a major cause of cancer-related death among women, accounting for 30% of female cancer cases. Despite advancements, the survival rates for High Grade Serous Ovarian Cancer (HGSOC) have remained consistently low over the past two decades. This underscores the urgent need to identify novel factors contributing to these malignancies. Intriguingly, no specific driver genes have been discovered for HGSOC so far. This indicates that HGSOC progression may not be governed by specific mutations and suggests that extrinsic cues could promote the tumour's development. Moreover, HGSOC is primarily characterised by genome instability, which has been associated with disruption of endoplasmic reticulum (ER) proteostasis.

AGR2, an ER-resident protein disulfide isomerase (PDI), is an unfolded protein response (UPR)-induced gene, a marker of ER proteostasis defects. Moreover, ER stress leads to the secretion of AGR2 into the extracellular milieu. We have shown that AGR2 is overexpressed in HGSOC and secreted into its tumour microenvironment. Our results demonstrate that extracellular AGR2 (eAGR2) in the HGSOC cellular microenvironment results in several tumour-associated processes: (1) it promotes cell proliferation, (2) increases overall protein synthesis, and (3) enhances vimentin expression, a key marker of epithelial-mesenchymal transition (EMT). Thus, eAGR2 exerts a gain of function in the tumour microenvironment by promoting pro-tumour activities that are independent of its ER function.

In conclusion, we have identified a novel mechanism by which ER proteostasis alteration could be signalled throughout the tumour cell. Our results identify the PDI family member AGR2, as a novel pro-oncogenic extracellular signalling molecule in HGSOC development.

P17 - Evaluation of PCR kits detecting macrolide resistance in Mycoplasma pneumoniae

Nadège Hénin | *MFP, U5234*

An outbreak of Mycoplasma pneumoniae infections took place in France in winter 2023. Macrolide are the first-line treatment but macrolide resistance has been reported. This study aimed to assess the clinical performances of two commercial kits for the detection of macrolide-resistant M. pneumoniae: Mycoplasma Pneumoniae and Macrolides-Resistant Strain Nucleic Acid Test Kit (Mole Bioscience (MB)) and LightMix® Modular Mycoplasma Macrolide (TIB Molbiol (TM)) in comparison with the 23S rRNA Sanger sequencing used as the reference.

Methods

A total of 237 ear, nose, and throat swabs and respiratory specimens were evaluated including 100 negative specimens, 100 wild-type (WT) and 37 mutated M. pneumoniae-positive specimens. DNA extraction was performed using MagNa Pure 96 (Roche) and both kits were run according to the manufacturer's instructions.

Results

The MB kit, but not the TM kit, was also designed to detect M. pneumoniae in specimens. However, M. pneumoniae was not detected in 27.7% of M. pneumoniae-positive specimens, hampering the subsequent detection of resistance by this kit. For the TM kit, 5.1% of positive specimens were not amplified. The clinical sensitivities for detection of macrolide resistance-associated mutations in clinical samples were 90.9% and 81.5% for the TM and MB kits, respectively. All false WT results were found in specimens harboring a 23S rRNA mutation at position 2067 (M. pneumoniae numbering). The clinical specificities were 97.9% and 94.4%, for the TM and MB kits, respectively. Using the MB kit, specimens with high M. pneumoniae loads were detected as falsely mutated. When these specimens were 1/100 diluted, the expected WT results were recovered. Additionally, for the TM kit, melting temperatures from WT specimens were not within the temperature range provided in the manufacturer's instructions. The WT control provided in the kit was thus necessary for comparison and interpretation purpose.

Conclusion

A lack of sensitivity for M. pneumoniae detection was observed with the MB kit, hindering the detection of macrolide resistance with this kit. None of the two kits accurately detect macrolide resistance-associated mutations located at position 2067.

P18* - Impact of mitochondrial-associated cannabinoid receptor Type 1 (mtCB1) signaling on glioblastoma progression

Camille Humeau | *BRIC, U1312*

The cannabinoid receptor type 1 (CB1) is one of the most abundant G protein-coupled receptors in the central nervous system, playing a crucial role in regulating neuronal transmission and several key physiological processes. Interestingly, CB1 is not only found on the plasma membrane but is also functionally linked to mitochondrial membranes (mtCB1) in neurons and astrocytes, where it regulates oxidative and glycolytic metabolism.

Recent evidence suggests that CB1 plays a role in the metabolic activity of glioblastoma (GB), an aggressive and incurable form of brain cancer known for its high metabolic adaptability. While cannabinoids have been shown to inhibit GB growth and invasiveness, the mechanisms driving these effects remain unclear. CB1 agonists, in particular, have been observed to induce apoptosis in cancer cells, yet their impact on mitochondrial function and tumor metabolism is still not well understood.

Preliminary findings indicate that mtCB1 is present in GB cells, where it controls energy production needed for cell invasion and proliferation. Targeting mtCB1 may offer a novel therapeutic strategy to disrupt the metabolic flexibility of GBM, opening new avenues for combatting this highly resistant cancer.

P19* - Study of probiotics effects on gastric carcinogenesis in the context of Helicobacter pylori infection

Marine Jauvain | *BRIC, U1312*

Background : The occurrence of gastric adenocarcinoma linked with Helicobacter pylori infection is influenced by different factors including the digestive microbiota. Lactic acid bacteria role on digestive carcinogenesis are discussed and some Lactobacillus species have been shown to act against H. pylori-induced inflammation and colonization, but their effects on H. pylori-related carcinogenesis have not yet been studied. The effects of Lactobacillus spp. on the epithelial-to-mesenchymal transition (EMT), cancer stem cells properties (CSC) and the inflammation in response to H. pylori infection were investigated.

Methods : A co-culture model was used with AGS cells infected with H. pylori associated with 20 different probiotic strains candidates. Different indicators of EMT and CSC properties were studied; including humming bird phenotype quantification, tumorsphere formation assay, ZO-1 and Integrin 1 expression with western-blot and immunofluorescence assays. Effect of the Lactobacillus spp. on the inflammation in response to H. pylori was also evaluated by quantifying IL8, IL6 and TNF production with ELISA assay.

Results : Among the strains tested, a significant decrease of the humming bird phenotype induced by H. pylori was observed in presence of Lactobacillus gasseri and Lactobacillus rhamnosus with a reduction of 65% and 70% respectively ($p<0.0001$). L. gasseri and L. rhamnosus also decreased the number of tumorspheres formed (reduction of 24% and 50% respectively, $p<0.001$). IL-8 production was also significantly reduced in presence of L. gasseri and L. rhamnosus.

Conclusion : These results suggest that L. rhamnosus and L. gasseri may have an inhibitory effect on carcinogenesis induced by H. pylori.

P20* - B-Catenin Phosphorylation by AKT at Serine 552: A Novel Mechanism for CDT Modulation

Ruxue Jia | *BRIC, U1312*

Some strains of bacteria can produce toxins, such as colibactin and cytolethal distending toxin (CDT). These toxins induce DNA damage and inflammation, well-established risk factors for cancer, particularly colorectal cancer. The Wnt/B-catenin signaling pathway plays a crucial role in cancer development and progression, driving tumor initiation, growth, and metastasis.

In the present study, we examined the impact of the cytolethal distending toxin active subunit, CdtB, on the Wnt/B-catenin pathway in human intestinal and hepatic epithelial cell lines.

CdtB exposure resulted in the loss of adherens cell junctions (B-catenin and E-cadherin). Additionally, CdtB exposure increased the phosphorylation of B-catenin at serine 552. This phosphorylation correlated with B-catenin's nuclear localization and its enhanced transcriptional activity, as confirmed by microarray analysis, RT-qPCR analyses and TOP/FOP-Flash luciferase reporter assay.

Metformin, a commonly prescribed oral medication for type 2 diabetes, inhibits the phosphorylation of B-catenin at Serine 552 through the AMPK/PI3K/AKT pathway. This compound mitigated the CdtB-induced loss of B-catenin at cell-cell junctions, decreased B-catenin phosphorylation at Ser552, and reduced its nuclear accumulation and transcriptional activity. These findings were further supported by similar observations with MK2206, a highly selective and allosteric direct inhibitor of AKT.

Overall, these findings indicate that CdtB activates the AKT signaling pathway, leading to the phosphorylation of B-catenin at Ser552. This phosphorylation facilitates B-catenin's dissociation from cell-cell junctions and subsequent nuclear translocation, preventing cytoplasmic degradation, and ultimately resulting in increased B-catenin transcriptional activity.

P21* - Linezolid penetration in cerebrospinal fluid of mouse: impact of bloodbrain barrier disruption

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Introduction: Linezolid is used to treat central nervous system infections caused by methicillin-resistant Staphylococcus due to its favourable penetration into the cerebrospinal fluid (CSF). The mechanisms involved in this penetration are not completely understood. The aim of this study was to assess the impact of blood-brain barrier (BBB) disruption on CSF penetration of linezolid.

Material and methods: Thirty C57bl6/J mice were divided in two groups of 15 animals. One group received intraperitoneally a single dose of linezolid (40mg/kg) at T0. The second group received 10mg/kg of lipopolysaccharide (LPS) 30 minutes before the linezolid administration. Three mice from each group were sacrificed 30 minutes, 1h, 2h, 4h and 8h after linezolid administration. BBB permeability was evaluated at each sampling time by immunohistochemistry using immunoglobulins diffusion through vessels in brain tissue. A LC-MS/MS method was developed and validated to measure total linezolid concentrations in CSF and plasma. The penetration ratio of linezolid from the blood to CSF was calculated by the

AUC0-8h (area under curve) ratio (AUC0-8hCSF/AUC0-8hplasma).

Results: Immunoglobulins diffusion through vessels in brain tissue was significantly increased in LPS-group at each time point, with a maximum 8 hours after LNZ administration: 1817µm2 in LPS-group and 58µm2 in LPS-free group (mean). The mean Cmax of linezolid was 40,263ng/mL and 41,613ng/mL in LPS-and LPS-free groups, respectively. AUC0-8h in LPSfree group were 120,607ng/mL*h and 38,868 ng/mL*h in plasma and CSF respectively. In LPS-treated group, AUC0-8h were 191,805ng/mL*h and 60,680ng/mL*h in plasma and CSF, respectively. AUC0-8h ratios were 32.2% for LPS-free group and 31.6% for LPS-treated group.

Discussion / Conclusion: This study successfully developed a mice model to assess CSF penetration of linezolid in conditions of BBB disruption.The BBB opening does not appear to influence the penetration of linezolid in CSF. It would be interesting to investigate therole of efflux transporters, such as P-gP or BCRP in linezolid penetration in CSF.

P22* - Investigating the Role of Gut Microbiota in Glioblastoma Development

Sarah Lavielle | *IBGC, U5095*

Glioblastoma is the most common and aggressive brain tumor in adults, characterized by a poor prognosis, with a median survival of approximately 14 to 15 months and frequent therapeutic failure. Improving patient survival and quality of life requires identifying factors that contribute to its initiation, progression, and therapeutic resistance. This study focuses on the gut-brain axis, specifically the potential role of the bacterial microbiota in glioblastoma progression. The bacterial microbiota refers to the diverse community of bacteria that colonizes the gut, which has been shown to play a crucial role in several brain pathologies, including Parkinson's disease and Alzheimer's disease. Recent studies suggest that the microbiota may influence glioblastoma, but the underlying mechanisms remain unclear.

This project has two main objectives:

1. Evaluate the effect of bacterial microbiota depletion on glioblastoma progression.

2. Investigate how gut bacteria influence glioblastoma by studying tumor immune modulation in vivo and the impact of gut-derived metabolites on glioblastoma stem cells in vitro.

To achieve this, C57BL/6 mice were implanted with murine glioblastoma stem cells (mGB2) and treated with antibiotics to deplete their gut microbiota. Tumor growth was monitored by bioluminescence imaging, and immune cells infiltration into the tumor was analyzed using RNAscope multiplex staining. The effect of two metabolites, serotonin and butyrate, was tested in vitro on murine (mGB2) and patient-derived (P3) glioblastoma stem cells to assess their impact on invasion and proliferation.

Results showed reduced tumor progression in microbiota-depleted mice, with a significant decrease in immune cells infiltration. In vitro, serotonin increased glioblastoma cell proliferation but reduced invasion, while butyrate decreased proliferation and increased invasion.

These findings suggest a role of bacterial microbiota in glioblastoma progression and may help identify new therapeutic strategies targeting microbiota to improve patient outcomes.

P23 - In Vitro Selection and Characterization of Resistance to Josamycin and Pristinamycin in Mycoplasma genitalium

Chloé Le Roy | *MFP, U5234*

Macrolide resistance has emerged in Mycoplasma genitalium. Pristinamycin is part of the recommended third-line treatment according to the European guidelines. No data regarding mechanisms of pristinamycin resistance have been available to date. In M. pneumoniae, a phylogenetically close species, cross resistance to pristinamycin and josamycin was associated with mutations in the 23S rRNA gene at position 2062. We investigated in vitro development of resistance in M. genitalium in the presence of subinhibitory concentrations of josamycin and pristinamycin.

Selection of resistant mutants was performed by serial passages of M. genitalium G37 reference strain in FRIIS medium containing subinhibitory concentrations of josamycin or pristinamycin. Resistant mutants were characterized by PCR amplification and Sanger sequencing of 23S rRNA, L4 and L22 ribosomal protein genes. For each resistant mutant, MICs of seven antibiotics were determined. Whole genome sequencing (WGS) was then performed on selected mutants using the Illumina technology.

A mutant selected in the presence of josamycin harbored an A2059G mutation in 23S rRNA. This mutant showed a strong increase in the MICs of erythromycin, azithromycin, josamycin, and clindamycin, but no changes in MICs of pristinamycin, doxycycline, and moxifloxacin.

Two mutants selected in the presence of pristinamycin harbored the mutation A2062C or A2062G. Both mutants showed a strong increase of the MICs of pristinamycin and josamycin and a slight 4 to 8-fold increase of erythromycin MIC.

WGS of the three mutants confirmed the 23S rRNA mutations associated with antibiotic resistance and revealed 6 to 10 additional SNPs per mutant compared to the reference strain sequence. These SNPs were located all around the genome and were not likely to be involved in resistance.

This study showed that resistant mutants can be selected in vitro in M. genitalium using josamycin and pristinamycin. These laboratory-derived mutants could be predictive for mutations observed in clinical strains.

P24* - Slit/Robo signaling in glioblastoma invasion

Teo Leboucq | *BRIC, U1312*

Glioblastoma (GBM) is the most common malignant brain tumor worldwide. Blood vessel development (angiogenesis) drives GBM progression. Blocking a single angiogenic growth factor VEGF slows GBM progression in patients, demonstrating that anti-angiogenic therapy is a viable option to inhibit tumor growth. However, tumors evade inhibition suggesting that other factors besides VEGF must drive tumor vascularization. In addition, tumor cell infiltration contributes to tumor recurrence.

We have identified the guidance factor Slit2 as a novel angiogenic factor in mice. Moreover,

we lately demonstrated, in glioma context, that Slit2 promotes myeloid cell recruitment in the tumor microenvironment, which in turn abnormalizes blood vessels morphology and function, supporting tumor progression.

Lately, we evidenced that tumor cells themselves express Robo receptors at the leading edge of the tumor invading front. We propose that Slit2 may also affect, directly or indirectly, glioma cells infiltration.

Our study shows the chemoattractant effect of Slit2, via Robo receptors, on patient derived tumor cells in vitro and in vivo. In vitro, Slit2 promotes orientated migration and tumor spheroid invasion via Src kinase signaling, effects which is lost in tumor cells depleted for their expression of Robo receptors using an anti-sens strategy. In vivo, the depletion of Robo receptors induces an impressive tumor growth delay of patient derived tumor cell spheroid implanted orthotopically in Raggamma mice, which might be explained by direct invasion miss-guidance, but also an alteration of the full invadosome mechanism, as suggested by a downregulation of MMP signaling in Robo depleted tumor cells which are required for matrix degradation prior invasion.

This study provides the first comprehensive examination of Slit-Robo-signaling in GB cells, with the goal to develop innovative therapy strategies to prevent GB invasion.

P27* - Role of LAV-BPIFB4 in Glioblastoma: new insights

Valentina Lopardo | *BRIC, U1312*

Glioblastoma multiforme (GB) is classified as a grade IV glioma and it is the most prevalent malignant primary brain tumor, with a 5-year survival of just 7.2%. The standard-of-care for GB patients typically involves surgical resection followed by radiotherapy in combination with temozolomide (TMZ)-based chemotherapy.

Recent findings indicate that the longevity-associated variant encoded by a four-SNP haplotype of BPIFB4 gene (LAV-BPIFB4) plays a role in shaping the immuno-senescence and pro-inflammatory microenvironment in GB patients, along with a senolytic effect on GB cell lines. This variant, reducing TMZ-related senescence, chemosensitize cancer cells.

New data highlight a metabolic effect of LAV-BPIFB4, able to affect the bioenergetic capacity of cancer cells, by reducing the mitochondrial oxygen consumption. Using in vitro 3D models, we showed that LAV-BPIFB4 is able to impair the tumor invasiveness of patients derived GB stem-like cells regardless of their molecular signatures. These preliminary results led us to speculate a new mechanism of action of the LAV-BPIFB4 on the mitochondrial energy production, required for tumor invasiveness and progression.

P28* - Intestine-on-chip as model to study infections by Candida yeast

Fernanda Lopez Garcia | *MFP, U5234*

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. Organs-on-chips (OOCs) are an alternative to model organ functionality and recapitulate some of their physiological or pathological features in vitro. 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 (~µm) resolution but fast and label-free acquisition is envisioned. We aim to provide an in-depth investigation of the mechanisms underlying intestinal infection by Candida yeast with the perspective of identifying new routes for therapeutic treatments. The Intestine-on-chip consists of a microfluidic chip with 2 micro-channels separated by a central porous membrane, on either side of which epithelial cells and vascular endothelial cells will be adhered, mimicking the interface of a vascularized human organ. Two lateral vacuum channels allowing the generation of mechanical stretching of the membrane will be included to mimic in vivo intestinal cells environment.

P29 - Deciphering the oncogenic properties of Fascin-1 in Hepatoblastoma

Grégoire Manaud | *BRIC, U1312*

Hepatoblastoma (HB) constitutes the most common form of pediatric liver cancer, accounting for 1% of all malignancies in children. HB treatment is a combination of chemotherapy and surgical resection of the liver segments affected by the tumor. Despite good efforts leading to an 80% survival at 5 years, side effects are observed in children and negatively impacting their quality of life as well as their long-term outcomes. A distinctive genetic hallmark of HB is the high rate of CTNNB1 mutation found in 89% of cases, leading to an aberrant activation of the Wnt/B-Catenin pathway, and make it attractive as a targeted therapy for HB. However, giving the high risks of side effects, we aim to identify new B-Catenin dependent targets. In this aim, we propose to use Fascin-1 encoded by the FSCN1 gene, found to be a transcriptional target of B-Catenin and upregulated in HB. Fascin-1 an actin-bundling protein localized in filopodia and thus promoting cell migration. As such, Fascin-1 is expressed in progenitors but remains absent in most of mature differentiated cells. Interestingly, we found that Fascin-1 expression was upregulated in a subset of HB with a poor prognosis characterized by the presence of undifferentiated and highly proliferative cell clusters. We demonstrated that indeed, Fascin-1 expression is correlated with hepatocyte differentiation status. To explore the underlying mechanisms, we have built the hypothesis that the cellular localization of Fascin is responsible for the alteration of hepatocyte differentiation. We use the B-Catenin-mutated HB cell lines HepG2 and Huh6 and we observed that the phospho-mimetic Fascin mutant S39E increase YAP expression and we propose that it stimulates the gene expression related to hepatocyte undifferentiated status in vitro. Thus, our results suggest a key role of Fascin-1 in HB progression and that Fascin-1 may represent a new therapeutic target in HB.

P30* - Microtubule and centrosome remodeling in quiecence

Aurélie Massoni-Laporte | *IBGC, U5095*

Cells are constantly facing decision to proliferate or enter in a non-proliferating state. Quiescence is defined as a temporary absence of proliferation, and is the most widespread cellular state on Earth, ranging from prokaryotic to eukaryotic organisms. Quiescence establishment, maintenance and exit, by balancing cell proliferation, are key steps involved not only in normal development and tissues homeostasis, but also in major human pathologies such as cancers. Quiescence is at the heart of the aging process, as over time cells must overcome the deleterious effects of constant increase in damaged macromolecules while maintaining their ability to reproliferate.

Recently, it has been shown that microtubules, key cellular structures for chromosome segregation, are reorganized and stabilized in quiescent yeast cells. This novel structure, named Q-nMT bundle, is required for the survival of quiescent cells, and is involved in controlling the return to the proliferative state.

We have investigated the relationship between the Q-nMT bundle and the centrosome from which it originates. I have shown that the yeast centrosome is modified upon quiescence establishment, with an increased recruitment of gamma- TuRC components. . We are currently searching for possible post-translational modifications of gamma-TuRC and partners that may be involved in Q-nMT bundle formation. Finally, upon exit from quiescence, I have shown that the Q-nMT bundle disassembly is mandatory for centrosome separation upon re-entry into mitosis. We propose that, like primary cilia in metazoans, the Q-nMT bundle may act as a checkpoint to allow quiescence exit, thereby opening new avenues for understanding the interdependence between the primary cilium and quiescence.

P31 - Role of immunosuppressive myeloid cells on gastric cancer stemness promotion

Tra-Ly Nguyen | *BRIC, U1312*

Gastric cancer (GC) is the 4th leading cause of cancer-related deaths globally. Our research has identified cancer stem cells (CSCs), including a mesenchymal subpopulation found as metastatic circulating tumor cells (CTCs), which drive tumor initiation and chemoresistance in GC.

CSCs are a small but critical group of cancer cells marked by CD44 expression, driving tumor growth, chemoresistance, and metastasis through epithelial-to-mesenchymal transition (EMT). We've identified a highly invasive mesenchymal-like CSC subpopulation expressing CD44v3/CD44v6, detectable as circulating/metastatic tumor cells and possibly metastasis-initiating cells (MICs). Research suggests these cells exist as single cells or clusters, often associated with immune cells like neutrophils or myeloid-derived suppressor cells (MDSCs), which facilitate metastasis. However, the interactions within CTC/MIC-immune cell clusters and their role in metastasis remain unclear.

Project Overview: This project investigates how MDSCs influence the tumorigenic and invasive properties of gastric CSCs, particularly MICs. We co-cultured GC cell lines with human monocyte-derived suppressor cells (HuMoSCs), an MDSC model, and found that HuMoSCs enhance stemness in GC cells via contact-dependent mechanisms, as seen in tumorsphere and transwell assays. Flow cytometry showed an increase in CD44v6+ cells, and

immunofluorescence revealed elevated ZEB1 nuclear localization. In vivo models also suggest HuMoSCs might contribute to tumor formation.

Conclusion: Our preliminary data indicate HuMoSCs promote tumorsphere growth and CSC features in vitro. These findings will be validated using CD33+ myeloid cells from GC patients, and scRNA-seq will help uncover the mechanisms driving cancer stemness. Ultimately, this project aims to develop therapies targeting CSC/MIC-MDSC interactions, providing new diagnostic and prognostic tools for GC.

P33* - Biophysical studies of G-quadruplexes from the 5' UTR of mRNA in KRAS and their regulation by nRNP-A1 protein

Zahraa Othman | *ARNA*

The untranslated region (UTR) of messenger RNA (mRNA) in proto-oncogenes plays a crucial role in gene expression regulation. These UTRs, situated at the 5' and 3' ends of the mRNA, contain regulatory elements that influence translation and stability. In proto-oncogenes, the UTRs can harbor regulatory sequences that control the expression of the corresponding oncogenes. Dysregulation of UTR-mediated processes can reduce oncogenic transformation by affecting translation initiation, protein production levels, be the target of microRNAs, impacting mRNA stability and degradation. Understanding the complexities of UTR regulation in proto-oncogenes is vital for uncovering mechanisms behind cancer development and potential therapeutic interventions. In this proposal, we want to address this problem in KRAS 5'UTR region, where the partner-laboratory from Udine recently discovered the formation of unusual structures (G-quadruplexes) that are the target of regulatory proteins, confirmed here in Bordeaux as well by biophysical methods. Three regions (UTR-1, UTR-C and UTR-Z) from 5' region where independently target with RNA binding protein (RBP) hnRNPA1. Preliminary results using biochemical and biophysical tests evidence a regulatory and strong interaction particularly with UTR-Z.

P34* - Telocyte: a new player in the regression of tumor vasculature? Example of infantile hemangioma

Léa Pechtimaldjian | *BRIC, U1312*

Infantile hemangioma (IH), is the most frequent tumor in newborns. This benign cutaneous vascular tumor undergoes a fast-growing phase followed by a slow and gradual regression. The team recently demonstrated that the telocyte (TC), a newly discovered stromal cell may play a role in the IH unique cycle. However, mechanisms of regression mediated by TCs, remain unclear. We thus wondered how TCs could contribute to this process. Given their pivotal roles in cellular communication and tissue organization, we hypothesized that TCs could participate in IH involution by inducing a vascular remodeling.

Assessing the remodeling of vessels requires large-volume imaging. We thus developed an advanced quantitative and spatial imaging of millimeter-thick portions of cutaneous tissue, named Skin-iDISCO+. In contrast with other tissue-clearing protocols, we provided a method enabling the imaging of pigmented and matrix-dense tissue like the human skin followed by the staining of capillaries and TC networks. Their bioinformatic reconstructions allow morphometric parameters extraction to provide quantitative outcomes. Skin-iDISCO+, on both proliferative and involutive patient resections enable us to identify that lesion capillaries have

undergone normalization upon involution. Moreover, that phenomenon correlated with the perivascular TC plasticity and morphological switch.

To better understand whether TC switch was “cause or consequence” of vessel normalization we developed the first patient-derived 3D model of IH including TC. Thanks to this dynamic model we assessed the angiogenic potential of TC on pseudovascular structures formed by endothelial cells. Results highlighted the specific and paracrine anti-angiogenic activity of TCs from involuting patients. A preliminary 55 molecules screening gives rise to a specific anti-angiogenic signature.

To conclude, thanks to our double approach of large-volume imaging and patient-derived 3D model we identified TC as an important effector on capillaries homeostasis. Targeting their morphological and functional switch could offer an interesting alterative in favor of the tumor vasculature normalization.

P35* - Impact of DDR1 on Renal Cell Carcinoma development

Chloé Redouté-Timonnier | *BRIC, U1312*

Renal Cell Carcinoma (RCC) accounts for 90% of kidney cancer cases, with clear cell RCC (ccRCC) being the most common subtype, comprising 75% of cases. In 2018, over 350,000 new RCC cases were reported worldwide. Current ccRCC treatments involve a combination of tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors. Among the TKI targets is discoidin domain receptor 1 (DDR1), a collagen-activated receptor tyrosine kinase.

Interestingly, analysis of TCGA data suggests higher DDR1 expression correlates with better survival outcomes in ccRCC patients, indicating DDR1 may have a protective role by inhibiting tumor progression. To investigate the role of DDR1 in ccRCC, we engineered Renca and 786-0 ccRCC cell lines to overexpress or knock out DDR1. In vitro assays assessing cell proliferation, migration, and collagen invasion revealed that DDR1 overexpression led to a collagen-dependent reduction in proliferation, marked by G1/G0 phase arrest and decreased S phase entry. This was linked to increased expression of cell cycle inhibitors p21 and p27, and decreased expression of CDK1 and CDK2.

DDR1 overexpression also resulted in reduced collagen-dependent migration in 786-0 cells, with a similar trend observed in Renca cells. In Renca cells, the expression of EMT markers Snail1 and Zeb2 was downregulated. The Phospho-kinome analysis (PamGene technology) indicated that DDR1 activation decreased intracellular activation of the pro-survival factor AKT.

In vivo studies using orthotopic mouse models of ccRCC showed a significant reduction in tumor growth with DDR1 overexpression.

Overall, these data showed that DDR1 activation reduces proliferation and migration of ccRCC cells by modulating key proteins of the cells cycle, gene expressions involved in EMT and AKT activation. Consequently, ccRCC development is impaired. As a next step, we aim to investigate the mechanisms responsible for DDR1 downregulation in ccRCC, which may offer the identification of novel therapeutic targets to improve treatment outcomes.

P36* - Lentivector onco-targeting for solid tumor gene therapy

Julia Rossi | *BRIC, U1312*

Among the innovative therapeutic options in cancers refractory to current therapies such as pancreatic adenocarcinoma and metastatic prostate cancers, gene-based therapies show considerable promise. First, cancer gene therapy needs efficient gene transfer into the tumors. Lentiviral vectors pseudotyped with the broad tropism VSV-G envelope glycoprotein provide high transduction efficacy. Second, cancer gene therapy needs oncospecific transfer of the therapeutic genes, especially when bringing potentially harmful signals for healthy cells.

To obtain tumor-restricted oncotropism, we compared several pseudotyping solutions targeting cell surface antigens of pancreatic and prostate cancer models. We produced oncotropic lentiviral vector by engineering the E2 recognition glycoprotein of Sindbis virus (SINV-G), leaving intact the fusion E1 monomer. The scFv-SINV-E2 showed as efficient as the broad tropism VSV-G, but with exclusive specificity to cells expressing the targeted antigen. Moreover, with these envelope glycoproteins, the transduction efficiency was proportional to antigen expression by cancer cells, a crucial point when healthy cells display low expression of the targeted antigen. Intra-tumor injections of lentiviruses displaying scFv-SINV-E2 in mice bearing subcutaneous tumors produced transduction rates as efficient as lentiviruses displaying VSV-G. Importantly, while VSV-G lentivectors injected intravenously delivered the reporter gene at the injection site, in the liver, and in the bone marrow, scFv-SINV-E2 lentivectors reached only the tumors and its metastasis and no other detectable site, confirming the strong therapeutic anti-cancer value of lentivector onco-pseudotyping.

In conclusion, we confirmed oncotropic vectors engineered with antibodies or their variable fragments target only the cancer cells, with high reproducibility and applicability for in vivo transfer of therapeutic genes. Several modalities for viral glycoprotein engineering highlight the adaptability of lentiviral targeting to any tumor cell surface landscape. Considering the difficulty of reaching every tumor cell with toxic genes, this onco-specific targeting will be key for implementing intratumoral vulnerabilities.

P37* - Study of a new variant of FLT3 in Acute Myeloid Leukaemia

Claire Rouy | *BRIC, U1312*

Acute Myeloid Leukaemia (AML) is characterised by a blockade of differentiation and an increased proliferation of immature myeloid cells named myeloblasts in the bone marrow and in the blood. These leukemic cells carry different genetic alterations, among them mutations on the FLT3 gene. Almost 30% of AML patients have an internal tandem duplication (ITD) mutation in the juxta-membrane domain of FLT3 or mutations in tyrosine kinase domains (TKD) leading to constitutive activation of this tyrosine kinase receptor. These mutations are often involved in relapse and resistance to conventional treatment. That is why the ITD mutation, which is associated with poor prognosis, was identified as an important therapeutic target. Several anti-FLT3 tyrosine kinase inhibitors (TKI) are currently used in clinics but resistance often appears.

In this project, our group identified a new splicing variant of FLT3 in an AML resistance context. It has been identified in AML patients at diagnosis from a cohort of Bordeaux. The objective of this project is to characterise this new variant.

The oncogenic potential through proliferation and signalling analysis have been characterized

in vitro using Ba/F3 cell line transduced with different vectors expressing our new FLT3 isoforms or other known FLT3 mutations. All together, these results suggest similarities between our new FLT3 variants and FLT3-ITD mutation even at their cellular localisation level. In addition to these in vitro results, the cell lines were injected into mice to validate the oncogenic potential of this new variant.

Furthermore, the effect of anti-FLT3 inhibitors, such as Midostaurine®, Gilteritinib®, and Quizartinib® has been assessed on the new FLT3 variants. The inhibitory effects of anti-FLT3 TKI, observed on the cell lines expressing known FLT3 mutations, are not found for cell lines expressing our new isoforms. These results strongly suggest that our new isoforms could be involved in resistance to TKI treatment.

P38* - Establishing a 3D Model to Investigate Hepatocellular Carcinoma Progression

Lucile Rouyer | *BRIC, U1312*

Hepatocellular carcinoma (HCC) is a highly proliferative cancer that develops over an extended period on a pathological liver. It is not clear how the surrounding liver tissue control HCC progression. HCC 3D models emerge as promising tools to study molecular mechanisms of HCC progression.

In this context, the aim of this project is to recreate an HCC 3D model recapitulating the main features of the disease including the tumor and the surrounding normal liver to decipher mechanisms involved in HCC proliferation.

We develop a co-culture 3D model using HepaRG and HCC tumor cells. We recreate the interface in a spheroid organized as tumor surrounded by healthy cells. This model maintains a good viability and structure during 2 weeks. The proliferation of tumor cells is reduced in the co-culture model compared to Huh7 monoculture spheroid and is inversely correlated with the increase number of healthy cells around the Huh7 spheroid. The proliferation of tumor cells was assess using live microscopy during 10 days and IHC (at different time points). We put in evidence that the contact between normal cells and tumor cells is require for proliferation decrease. The next step will be to modulate target proteins involved in HCC progression extracted from HCC tissues surgical resections and evaluate their impact on tumor progression in the model.

Our long-term objective is to facilitate the testing of potential therapeutics targeting critical aspect of HCC carcinogenesis and enhance our understanding of this complex cancer.

P39* - Bacterial genotoxins trigger invadosome formation associated to matrix degradation

Mariana Saraiva | *BRIC, U1312*

We are frequently exposed to bacterial genotoxins, such as Cytolethal Distending Toxin (CDT) and colibactin, produced by bacteria from the microbiota. These genotoxins cause DNA damage and a high degree of ploidy in host cells, well-known risk factors for carcinogenesis, along with stress fiber formation and cytoskeleton remodeling. We observed circular F-actin structures following exposure to bacterial genotoxins that may correspond to invadosomes, whose ability to degrade matrices contributes to invasion and metastasis. In this study, we investigated the mechanism of invadosome formation in response to bacterial genotoxins, Helicobacter hepaticus' CDT and Escherichia coli's colibactin.

In vitro, the immunostaining of invadosomes' markers in hepatic cell lines infected with genotoxin-producing bacteria, allowed the confirmation of invadosome induction. The increase in invadosome formation was dependent on the CDT and colibactin, as it was not observed in non infected cells and in response to the corresponding mutant strains invalidated for these toxins. Extracellular matrix (ECM) degradation was increased following exposure to these genotoxins. Similar results were observed when using transgenic cell lines expressing the CdtB catalytic subunit of CDT. A global kinase activity assay confirmed the CdtB-dependent activation of Src-family kinases, crucial in invadosome formation, and this was corroborated using a Src-family kinases inhibitor.

Overall, these data show that bacterial genotoxins lead to invadosome formation and ECM degradation, suggesting that chronic and/or repeated exposure to genotoxin-producing bacteria could be implicated in tissue remodeling and cancer progression.

P40* - Targeting B-lactamase activity with modified oligonucleotides to fight against antibioresistance

Lisa Scillia | *MFP, U5234*

Antibioresistance represents one of the main issues in public health since 1980s. Enterobacteriacae secreting extend spectrum B-lactamase are resistant to 3rd generation cephalosporine, the most used antibiotics in the world. Secreting ESBL's such as Escherichia coli, are at the top of the list of concern for the WHO, which classified them as a critical priority for R&D. Our main objective is to increase sensitivity to C3G in Escherichia coli resistant strain with the use of modified anti-sens oligonucleotides (ASO). This strategy is based on the principle of translation inhibition with oligonucleotides. These oligonucleotides are design to be complementary and anti-sens (ASO) to blaCTX-M-15 mRNA the gene coding for CTX-M-15 the most prevalent BLSE in Europe. ASO are chemically modified to increase their stability towards intracellular nuclease and improve their affinity for its target (PTO, LNA gapmer, etc,.). The principal barrier to the project is the crossing of the double membrane by the ASO. To improve the entry into the cytoplasm a nucleolipid is added in 5' ends (LASO) Preliminary results show that 5µM LASO PTO decrease ceftriaxone MIC from 2048 to 96mg/L in Ec3536 a clinical strain and from 1463 to 56 mg/L in TcK12 a laboratory strain secreting CTX-M-15. The present works is i) to confirm MIC experiment, ii) to demonstrate LASO internalization (high resolution microscopy and confocal microscopy), iii) to study impact of LASO at the transcriptional and translational level of CTX-M-15 (WB, proteomics, in vitro translation).

P41* - Role of carbohydrate-binding proteins in controlling glioblastoma stem cell fate and tumorigenesis

Myroslava Sliusar | *BRIC, U1312*

Introduction. Carbohydrate-binding proteins, galectins, are the family of proteins that specifically bind the B-galactoside sugars. Galectins (GAL, LGALS) are known to be associated with cancer development, including glioblastoma (GBM). Galectins can be considered worthy investigation targets for developing novel clinical approaches for GBM treatment.

Methods. Publicly available RNA-seq cohorts were analyzed with SUMO software and R. For in vitro experiments we used patient-derived glioblastoma stem cells (GSCs) BTSC73 and BTSC12. To study therapy resistance, GSCs were irradiated with 2 and 4 Gy. To reach hypoxic conditions, 5% CO2 and 1% O2 were maintained. The siRNA silencing was applied to achieve LGALS3 depletion. Live and apoptotic cells were counted with Annexin/PI assay. EdU cell proliferation assay was used to investigate cell proliferation.

Results. We showed that the simultaneous high expression of LGALS1, -3, -8, and -9 leads to the worst prognosis for patients over other combinations in glioblastoma RNA-seq TCGA datasets. Further scRNA-seq data analysis showed that Gal3 is extensively distributed among cancer cells, macrophages, and T-cells. The siRNA LGALS3 depletion in BTSC73 indicated a decrease in the number of live cells, which is related to the reduction of proliferation and not apoptosis. The siLGALS3 transfection decreases the level of stem cell markers Sox2 and Nestin. Furthermore, we detected an increase in LGALS3 and LGALS9 expression during differentiation. We also showed that hypoxia in contrast to irradiation, increases the LGALS3 expression.

Conclusions. GAL-1, GAL-3, GAL-8, and GAL-9 are greatly expressed during GBM, while simultaneous high expression of these galectins is associated with poor patient survival. Both Galectin-3 and -9 might be involved in the regulation of GSC differentiation. Galectin-3 could be involved in cancer stem cell regulation by the control of proliferation. Gal-3 also affects the level of GSC markers Sox2 and Nestin. Besides, Galectin-3 expression is regulated by hypoxia.

P42* - Zip Editing: an easy-to-use tool to increase CRISPR-Cas9 HDR-editing efficiency

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CRISPR-Cas9 holds great promise in the future of genome editing. Cas9 nuclease is very efficient to introduce double-strand breaks that are repaired by end-joining pathways resulting in insertions and deletions (InDels) or by homology-directed repair (HDR) using an exogenous template to introduce a desired modification. The latter repair mechanism is less frequently used by cells because it occurs only in S-G2 cell cycle phases and depends in part on the availability of the exogenous template at the site of editing at the moment of the repair. To increase the presence of the HDR template delivered as ssODN, several solutions has been developed to import it with the RNP complex (Cas9 + gRNA) but mostly rely on modifications of the Cas9 nuclease to link the ssODN. We propose a new editing tool, called Zip-Editing (ZE) to import the ssODN template with the RNP complex that doesn't rely on Cas9 modification. It is also not based on the modification of the cell cycle, or of DNA repair mechanisms, which can have deleterious genotoxic effects. Thus, ZE can be used with commercially available Cas9s, gRNAs and ssODNs and can also be very easily adaptable to a new target to introduce any type of modification. We tested ZE to edit precisely different targets (eGFP, UROS, CFTR) in

several cell lines (HEK-293T, K562) and in primary cells (human foreskin fibroblasts, human pulmonary basal cells, human hematopoietic stem and progenitor cells). We reached an increase in HDR-editing efficiency up to 12-fold as compared to a condition where ssODN template is not imported with the RNP complex. These results are very encouraging to impose ZE as a new tool to precisely edit the genome and to be part of the gene editing toolbox to treat or model diseases.

P43* - Interferon cross-protection from viral infection in primary bronchial epithelia

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Introduction : Bronchial epithelia (BE) is the primary target for many respiratory viruses. The BE intrinsic immune response to such viral infections involves two key steps: pathogen detection and activation of signaling pathways that counteracts infection. A critical antiviral signalling pathway is the interferon response, which can provide transepithelial activation of a number of interferon response genes (ISG). In this work we investigate whether infection of bronchial epithelia with specific respiratory viruses can induce an interferon-mediated «antiviral state» that confers protection against subsequent infections by the same or different viruses. Our experimental model is primary bronchial epithelia grown at the air liquid interphase derived from lung brushes of adult and child donors. We are comparing three common respiratory viruses for infections: Rhinovirus A (RVA) and C (RVC) and Adenovirus 5. To understand the dynamic and regulation of intrinsic immune response we infect BE with primary virus and challenge with a marker gene expressing RVA. In parallel we measure the release of interferon type I & III, and subsequent ISGs expression. We also monitor cytokine release and transcriptional response of the epithelia using scRNA-Seq.

Results and conclusions: The respiratory viruses show different kinetics of infection in primary BE. The difference in infection kinetics results in differences of intrinsic immune response. BE were protected against subsequent viral infections, but the dynamic of the cross protection depends on previous viral infection. While interferon release patterns were virus-specific, the regulation of ISGs was more complex suggesting donor dependence. Our data highlight the need to consider both viral and host factors in understanding respiratory infections.

P46* - Modelling metastatic dormancy of cancer stem cells in gastric cancer

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Cancer stem cells (CSCs) are a key factor in cancer metastasis, the leading cause of cancer-related deaths. These cells can survive for extended periods in a dormant state, resisting conventional treatments and potentially leading to tumor recurrence. Despite the significance of this problem, the underlying mechanisms of CSC dormancy in gastric adenocarcinoma (GC) remain largely unexplored.

Our research aims to investigate the molecular mechanisms that regulate CSC dormancy in GC. We are developing in vitro models to study how extracellular factors like hypoxia and ATRA influence CSC plasticity, particularly their transition between dormant, proliferative, and invasive states.

Our preliminary results indicate notably a potential role of ATRA and hypoxia in GC dormancy mechanisms. GC cells under ATRA treatment or hypoxic condition showed a lower proliferation rate in both proliferation and colony-forming unit assays compared to the control. However,

no increase in cell death was observed, suggesting that the cells might be arrested and surviving. This was confirmed by flow cytometry which showed an increase in the percentage of cells blocked in the G0/G1 phase of the cell cycle. We also observed variations in the protein expression and localization of dormancy markers such as NR2F1 and p27 by western blot and immunofluorescence. In conclusion, these preliminary results are encouraging but require further experimentations, which are currently ongoing.

Finally, this research project aims to develop and validate original models for the study of dormancy in the GC. Our ultimate goal is to identify the molecular signature of dormancy, and to elucidate the underlying mechanisms of dormant metastatic CSCs, thereby opening new perspectives for improving the therapeutic management of GC.

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