PhD symposium of the Institute of Genetics and Development of Rennes

27 October 2023

Le Diapason
Université de Rennes - Campus de Beaulieu
35700 Rennes
France

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From the cell to the organism

Force balance at the centrosome

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The centrosome is the main microtubule organizing center and as such is a key regulator of cell polarity. Indeed, its position directs the distribution of microtubules and thereby orients the main intracellular transport axis. Interestingly it is believed that microtubules are also the structures that position the centrosome. Both pushing forces produced by their polymerisation and pulling forces by molecular motors have been shown to influence centrosome position. However the mechanism establishing their exact balance at the centrosome is unclear. In this work we used laser nanoablation of single microtubules to investigate their individual contribution to this force balance. Unexpectingly, we found that microtubule ablation did not perturb centrosome position, and, even more surprisingly, that the total disassembly of the microtubule network did not prevent the maintenance of centrosome at the center of the cell. We further found that centrosome centering is actually controled by the contractile flow of the actin network. The microtubules only seem to radiate from the position defined by the actin network. However, when centrosome is experimentally moved away from that central position, we noticed that microtubules tend to organise cell shape around this new position. Suggesting that two mechanisms act in parallel: one, directed by the actin network, that position the centrosome at the cell center, and one, directed by the microtubules, that organized the cell around the centrosome. This duality ensures the robustness of centrosome centering in adherent cells, but also opens some possibilities for specific decentering in response to defined external cues.

Keywords: Centrosome, Microtubule

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The transcription factor Shavenbaby/OvoL ; a new regulator of adult muscle stem cells

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Short-talk #1

To compensate for organs damage and cell death, adult homeostasis requires organ regeneration mediated by long-lived stem cells. In young healthy individual, skeletal muscles are regenerated by the activity of resident muscle stem cells (MuSCs), which are quiescent and, upon injury, become activated and differentiate into myoblasts to repair the muscle. While MuSC differentiation starts to be understood, little is known about the molecular mechanisms that regulate the early steps of their activation. To gain further insight into the regulatory networks controlling MuSCs activation, we performed a gene candidate screen, based on a transcriptomic dataset of developing *Drosophila* flight muscles . We identified the transcription factor Shavenbaby (TF Svb/OvoL) as a new potential MuSCs regulator. We found that Svb is expressed in larval muscle progenitors, and maintained in adult MuSCs. Consistently, we identified a new *svb(MuSC)-Gal4* enhancer that controls its expression in the muscle progenitors and adult MuSCs. Using this enhancer, we performed lineage tracing experiments and I found that the adult Svb-positive MuSCs are able to proliferate and generate differentiated myoblasts. Importantly, we found that *svb* loss of function in adult MuSCs, leads to a significant increase in both the number of MuSCs and the rate of myoblasts production, indicating that *svb* controls MuSCs activation. Moreover, we observed that the high number of MuSCs and their progeny's location coincides with a muscle deformation at the attachment sites. Based on these findings, we hypothesize that the high production of muscle progeny leads to a muscle deformation that may results in a muscle detachment from the myotendinous junctions. Altogether, these data indicate that Svb controls MuSCs activation to finely regulate muscle homeostasis. At the PhD syposium we will present our recent unpublished data on the functional analysis of Svb/OvoL in MuSCs activation and muscle repair.

Keywords: Stem cells, Muscle, Shavenbaby/OvoL, Cell activation, Drosophila

*Speaker

Role of the Ensconsin/Kinesin-1 complex in the oocyte microtubule network reorganisation

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Flash-talk & Poster #1

The building of cell-type specific microtubule (MT) arrays are key features that regulate cell shape, intra-cellular transport and cell division. Although it is established that these processes are regulated by the MT-Associated Proteins (MAPs) including motors such as kinesin and dynein, it is not clear how these players fulfil their function to reorganize MT cytoskeletons in space and time. In this study, we have used *Drosophila* oocyte as a model system to investigate how a polarized array of short MTs is shaped into long MTs required for cytoplasmic advection and oocyte polarization. Unlike Kinesin-1, that exhibit homogenous localisation in nurse cells and oocyte chamber, its activator Ensconsin (Ens/MAP7), is strongly enriched in the oocyte suggesting the presence of a specific mechanism that target this MAP from nurse cells to the oocyte through ring canals. Interestingly, an Ens variant with low affinity for MT is not enriched in the oocyte. Moreover, loss of Dynein severely impairs Ensconsin enrichment in the oocyte in agreement that Ensconsin-decorated MTs are transported from nurse cells in the oocyte. We also show that MT elongation is strongly compromised in *ens*mutant, in agreement with the MT polymerising ability of Ensconsin. Finally, we show that Ens-Kinesin-1 interaction is strictly required to fully activate Kinesin1 that generate MT streaming. Altogether our work suggests that dynein dependant transport of Ensconsin-labeleld MTs may promotes targeted enrichment of MTs with enhanced polymerising properties, in the oocyte chamber. We propose that this step could be a prerequisite for MT reorganisation by Kinesin-1 into MT streams required for advection. Altogether, our work may imply that specific MT cytoskeletons may be assembled locally by motor-dependant transport of MTs harbouring enhanced polymerisation potential associated with Kinesin-1 activating properties.

Keywords: Microtubules, Drosophila, Oocyte, Kinesin, Ensconsin

*Speaker

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Microtubule bending rigidity likely plays a regulatory role during the division of the C. elegans zygote

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Flash-talk & Poster #2

Mitosis allows the development of an organism by faithfully partitioning the genetic material and cell fate determinants from a mother cell to two daughter cells. Microtubules, which are semi-rigid and dynamic fibers, are important players of this process. Indeed, they constitute the mitotic spindle, a key structure for accurate chromosome segregation. Furthermore, the astral microtubules, emanating from spindle poles and growing towards the cell periphery, participate in setting mitotic spindle position, a key element for cell fate determination. At cell periphery, they can either grow and push against it or be pulled by cortical molecular motors when shrinking, generating opposite forces. While the contribution of microtubule dynamics in mitotic fidelity is well established, the one of microtubule bending rigidity, namely microtubule capacity to bend under compression, has never been addressed.

Using *Caenorhabditis elegans* zygote as an established model of cell division, we study how the targeting of ZYG-8^{DCLK1}, the sole nematode member of the Doublecortin family, could affect cell division fidelity. Indeed, neuronal studies showed that proteins of the Doublecortin family can regulate microtubule mechanics by binding on microtubules. Interestingly, upon both *zyg-8(RNAi)* treatment and *zyg-8* mutation (*zyg-8(or484)),* we measured a reduced precision in spindle positioning at the cell center during metaphase and increased spindle-pole-oscillation amplitudes during anaphase. These perturbations suggest a less-confined positioning of the mitotic spindle, which likely originates from a reduction of cortical pushing forces, due to less rigid microtubules. Besides, we measured an increase in metaphasic plate width and observed chromosome mis-segregation when we target ZYG-8. We thus currently investigate whether microtubule-rigidity deregulation could perturb the forces which group and maintain chromosomes within the metaphasic plate, possibly by affecting microtubule-mediated pushing-forces against the chromosomes.

Overall, our work sheds light, for the first time, on microtubule-rigidity regulatory role during mitosis that complements the one of microtubule dynamics.

Keywords: Microtubule rigidity, ZYG-8, Mitosis

*Speaker

Identification of force generators regulating cell shape and cell identity through a genetic screen of GAPs and GEFs in the Drosophila notum

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Poster #3

While the expression of the genetic program is instructing a cell its identity, biomechanical cues such as mechanical cues including matrix elasticity for example, could affect the cell identity. This questions the impact of cellular forces regulating cell shape on cell identity. The actomyosin cytoskeleton is a well-established force generator in the plane of adherens junctions (AJs). Indeed, actin and the non-muscle type II myosin (Myo II) have the property to organize into contractile minifilaments able to impose forces on cell cortex, hence membrane deformations. To perform its functions, actomyosin activity is tightly controlled by Rho. Rho exists in two states, the activated state and the inactivated state. This molecular switch is activated by GEFs (Guanine nucleotide exchange factor) and inactivated by GAPs (GTPase activating proteins).

The objective of this work is to identify GEFs and GAPs that regulate Rho activity and consequently actomyosin network contractility in *Drosophila* notum epithelial cells that may impact their shape in the plane of the AJs and their identity.

The notum of the *Drosophila* is composed of two different cell populations: the epidermal cells (EPI) and the sensory organ precursor cells (SOP) that exhibit a different cell shape in the AJs plane. A RNAi screening of the GAPs and the GEFs was performed using non-invasive confocal microscopy live imaging. Images were analysed in an automatic and unbiased manner using a python written program. This program is automatically segmenting the cells using CellPose pretrained cytoplasmic model to create masks allowing the quantification of Myo II ratios within the cells as well as the perimeter, the area, the number of neighbors and the shape of the cells. The results of the screening that led to the identification of candidates regulating Myo II ratios, cell shape, and cytokinesis will be discussed.

Keywords: Screening, Actomyosin, Force generators, Automatic quantifications

*Speaker

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Does the asymmetric poleward flux in the C. elegans zygote reflect a functionally polarized spindle?

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Flash-talk & Poster #4

Cell division ensures the equal segregation of the two sets of chromosomes in the daughter cells. The mitotic spindle, a macromolecular structure composed mainly of microtubule filaments, force generators and their regulators, is at the heart of this process. Interestingly, such equal parting of genetic material contrasts with unequal distribution of cell contents and fate determinants in asymmetric division.

The cell deploys various control and corrective mechanisms to ensure the fidelity of division, especially destabilizing erroneous microtubule-chromosome bindings. They rely on the component's dynamics and particularly the microtubule's ability to alternate growth and shrinking phases. A particular instance of this dynamic instability is the poleward flux – a growth at the kinetochores coordinated with a shrinking at the microtubule minus-ends, closest to the pole. It resulted in a net microtubule movement towards the spindle pole and was associated with the fidelity of the division; however, the underlying mechanism remains elusive.

Using fluorescence recovery after photobleaching and image processing, we recently demonstrated that such a flux also exists in the nematode C. *elegans*, although restricted to kinetochore microtubules. We hypothesized that this flux is powered by kinetochore microtubules sliding along spindle microtubules anchored at the centrosome. Interestingly, the nematode one-cell embryo division is asymmetric, and the spindle is subjected to unequal pulling on both ends. We found that the apparent fluxes of kinetochore microtubules differ between the two half-spindles. This imbalance appeared dependent on GPR-1/2$^{\mathrm{LGN}}$, which controls cortical pulling force. This result is surprising as previous studies report that anterior and posterior spindle halves, and centrosomes are quasi-identical. We instead hypothesize that unequal fluxes are due to a functional asymmetry linked to the imbalance of the forces undergone by the half-spindles.

*Speaker

Relation between cell proliferation and cell differentiation in the intestine of C. elegans

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Poster #5

Unravelling the relationships between cell proliferation and cell differentiation is a key question underlying organogenesis. While proliferation allows tissue growth, differentiation generates specialised cell types. These programs are usually considered as being inversely correlated, with cell cycle exit generally coinciding with differentiation.

To address the relationships between cell proliferation and cell differentiation *in vivo*, we took advantage of the very stereotyped divisions and differentiation steps that occur during the embryonic development of the *C. elegans* intestinal epithelium. The intestine is composed of 20 cells all derived from a single precursor, the E blastomere. Interestingly, intestinal organogenesis shows a temporal coordination between the proliferation and differentiation programs.

We first investigated whether the intestinal differentiation program is able to regulate proliferation. To achieve this, we inhibited the different aspects of the differentiation program, namely the expression of differentiation transcription factors, polarisation and apical brush border formation. We showed that polarisation and brush border formation do not control the number of intestinal divisions. Thus, it appears that terminal differentiation is not required to arrest intestinal cell proliferation. In contrast, the absence of the differentiation transcription factors induced a 10-30% increase in the number of intestinal cells. The supernumerary cells appear late in development and in the posterior part of the intestine. Thus, these transcription factors seem to be able to limit the late proliferation of a subset of intestinal cells.

We are currently investigating the effect of the proliferation program on differentiation. We found that a mutation that accelerates early intestinal divisions does not alter the timing of cell polarisation and brush border formation. We will complement this work by studying different mutants with intestinal hyperplasia or hypoplasia. In particular, we will induce the degradation of the cycle inhibitor CKI-1 to study the impact of hyperplasia caused by a cell cycle exit defect.

Keywords: Organogenesis, Cell cycle, Proliferation, Differentiation

*Speaker

Characterization of a novel organoid model for the study of lens ocular development and pathology

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Short-talk #2

The ocular lens is a transparent organ composed of two cell types. The Lens Epithelial Cell (LEC) can differentiate into Fiber Cell (FC) which will form the bulk of the lens. During their differentiation, the FC undergo organelle and nuclei degradation making them challenging to study in classical two-dimensional culture. Consequently, most studies about the lens pathology rely on animal models. However, various research teams have developed three-dimensional lens organoid models that facilitate the *in vitro* lens studies. Nevertheless, these models rely on embryonic or induced pluripotent stems cells and numerous growth factor for a long culture time, making them costly and challenging to generate. We recently developed a new three-dimensional lens organoid obtained from an immortalized LEC cell line. These organoids can be easily generated within a few days without necessitating costly growth factor. We characterized this novel model to determine if it recapitulates some aspects of the lens development, more specifically FC differentiation. Similarly to the lens organization, our model is composed of distinct regions. Through histological and regional transcriptomic analysis assisted by laser capture microdissection, we describe the cellular changes occurring during the organoid formation. We find that this model recapitulates specific characteristics of the lens. While only the external layer of the spheroid shows proliferative cells, the inner region undergoes cellular changes partially mimicking the FC differentiation such as a mitochondrial degradation, the expression of crystallin and other genes specific of the FC. Additionally, this model is transparent and possesses the ability to focus the light. Mis-regulation of cataract genes (e.g., Celf1) or cataract-inducing compounds can compromise this optical property.Therefore, this user-friendly, cost-effective model enables lens research on environmental or genetic factors.

Keywords: Eye, Lens, Organoid, 3'RNA-seq, Laser Capture Microdissection

*Speaker

B cell intrinsic IL2RB signaling dampens extrafollicular response by promoting regulatory function and repressing IFN response

Juliette Gauthier * [1], Céline Delaloy† [1], Maxime Maugendre [1], Laure Michel [1], Patricia Ame [1], Céline Nonn [1], Simon Leonard [1], Yoni Desvois [1]

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Flash-talk & Poster #6

IL-2 is one of the first cytokine discovered but its complex roles on T cell effector functions has shadowed its function on B cell responses. B cells transiently expressed IL-2 receptor upon activation but it is unclear whether all mature B cell subsets have an equal dependence upon IL-2 and how IL-2 dictates B cell fate. Using a novel model of mice lacking IL2RB signaling specifically in mature B cells, we found that IL-2 acts intrinsically on IL2RB expressing B cells to promote IL-10 expression and plasma cell differentiation upon BCR activation *in vitro*. In contrast, *in vivo*, our results underscored that IL2RB signaling in B cells contributes to dampen the early extrafollicular humoral responses following T-dependent (SRBC) and T-independent type 2 immunizations (NP-Ficoll). We identified a subset of spleen-resident B cells capable of shifting from pro-inflammatory response to regulatory response following the activation of IL2RB signaling. Mechanistically, we found that IL-2 and interferon (IFN) signaling pathways collaborate to synergistically amplify IL-10 production by B cells. The lack of IL2RB signaling favors type I and II interferon (IFN) signaling in B cells that could be responsible for the enhanced magnitude of the antibody response skewed to IgG isotypes in *Il2rb* KO mice. In addition, using the experimental autoimmune encephalomyelitis mouse model we showed that IL-2 signaling on B cell is essential for the protective B cell response that controls disease severity. Altogether we uncovered a physiological B cell intrinsic IL2RB signaling, crucial for specific B cells to acquire suppressive functions, preventing them from assuming an alternative inflammatory effector cell fate at an early time point of immune responses.

Keywords: Interleukin 2, B cells, Differentiation, Immunoregulation, Mouse model

*Speaker

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Exploring Adipose-Muscle Communication: Impact of Mature Adipocytes on Myogenic cells in vitro Development in Rainbow Trout

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Flash-talk & Poster #7

The highlighting of the endocrine and paracrine role of adipose tissues shows an impact on various biological processes including growth. While the communication between adipose tissue and muscle is the subject of growing number of studies in mammals, the same cannot be said for fish, where a significant knowledge gap persists. This study aiming to elucidate the cross-talk between adipose and muscle tissues in the context of the continuous muscle growth in rainbow trout. The growth of precursor muscle cells can be studied *in vitro*, especially in trout where studies show simultaneous *in vitro* proliferation and differentiation of cultured myogenic cells. While the adipogenic precursor cells (pre-adipocytes) are commonly differentiated *in vitro* and used to study development, mature adipocytes (MAs) are rarely employed in the literature due to their buoyant characteristics and poor survival in culture. To approach *in vivo* conditions, our study place these mature adipocytes in indirect coculture with myogenic trout cells. We show an influence of matures adipocytes on myogenic cells *in vitro* development with an improved proliferation and reduced differentiation compared to monoculture myogenic cells. These effects are also found with conditioned media from matures adipocytes suggesting the presence of soluble factors capable of enduring freezing while remaining active. The use of an insert to separate the two cell types indicates factors of a size below 0.4μm. We are therefore moving towards the identification of factor(s) involved in the observed effects.

Keywords: Interaction, Coculture, Myogenic cells, Matures adipocytes

*Speaker

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The secret life of yeast - the Bdf1 interactome in the sporulation process

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Poster #8

Many fungi, including yeast, respond to sudden adverse changes in environmental conditions by sporulation. This process of meiotic division leads to the protection of their genome in highly resistant cells called spores. This leads to a radical reorganization of chromatin, which needs to silence a transcription and achieve an ultra-compact configuration, in a manner that can be entirely and rapidly reversed. It is still unknown how the yeast spores reach this phenomenal state which allows them to survive in poor conditions for a very long time.
The spore chromatin is highly compacted and hyper-acetylated on H4. H4 acetylation is essential for spore viability. This observation is seemingly counter-intuitive because H4 acetylation is usually associated with transcriptionally active chromatin. How such a strong compaction of acetylated chromatin is achieved? One of the possible answers is the evidence of the high level of Bromodomain Factor 1 protein (Bdf1) inside the spore, which has not only strong specificity toward acetylated H4 but also plays an important role in the process of transcription activation.

Keywords: Bromodomain Factor 1, Interactome, Saccharomyces cerevisiae, Epigenetics

*Speaker

Measuring the impact of m6A methyltransferase complex on R-loops dynamics at paused promoters in Drosophila

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Flash-talk & Poster #9

RNA modifications, known collectively as the epitranscriptome, have emerged over the past decade as an important additional regulatory layer of gene expression. RNA modifications can control many aspects of RNA metabolismincluding pre-mRNA splicing, degradation, and translation, and their disruption has been associated with a wide range of physiological alterations, neurological diseases, heart failure as well as various cancers. The modification of N6-methyladenosine (m6A) is of particular interest because of its high conservation during evolution, its asymetric distribution along mRNAs in correlation with the corresponding functions and its involvement in a wide range of developmental processes. Although the role of the m6A methyltransferase complex (m6A MTC) in transcriptional regulation has recently been described in many organisms, the context-dependent molecular mechanisms by which m6A deposited on RNA modulate RNA polymerase II (RNAPII) activity remain incomplete. We recently demonstrated that m6A MTC complex stimulates the release of RNAPII from its paused state. Our new project aims to specifically assess in this context (i) how m6A affects the stability of RNA/DNA hybrids called R-loops at promoter proximal regions (ii) what are the novel players linking m6A marks to R-loop resolution (iii) how this mechanism can be correlated to RNAPII pausing and to a particular epigenetic context (iv) how affecting the regulation of R-loops by m6A can lead to developmental defects. We believe our results will shed new light on the molecular understanding of m6A RNA function in transcriptional regulation.

Keywords: M6A RNA, RNAPII pausing, R loops, Transcription

*Speaker

Notch signaling pathway transcriptional dynamic during the muscle development and regeneration.

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Poster #10

The regeneration of skeletal muscles relies on muscle stem cells (MuSCs). Under normal circumstances, these cells remain quiescent. However, in response to muscle injury, they are activated, proliferate, and migrate to the damaged muscle. Throughout this intricate process, the fate of MuSCs is regulated by the Notch signalling pathway. Despite being a seemingly simple pathway, it is intriguing to consider how Notch regulates and coordinates all these steps. We hypothesized that Notch targets expression could be modulated by Notch activity during myogenesis. To address this hypothesis, we aim to analyze the *Drosophila* MuSCs the regulation of Notch targets transcription. Specifically, we aim to monitor and quantify the transcriptional response to Notch, in real time, using the MS2-MCP method. We generated new reporter fly line in which the MS2 stem-loops are inserted downstream of the Notch responsive element. We first showed, in fixed tissues, by single molecule FISH that the NRE-MS2 reporter are specifically expressed in the MuSCs. We also validated that the NRE-MS2 reporter responds correctly to Notch over-activation. Finally, we expressed a MCP-GFP in the MuSCs population and showed that the MCP protein localizes at the transcriptional foci. Together these data validated that our newly generated transcriptional reporter is expressed in the MuSCs and respond to an increased activity of the Notch signaling pathway. We will use this reporter to decipher whether and how the transcriptional response to Notch guides the cell fate decision of the MuSCs during muscle development and repair.

*Speaker

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Role of septate junctions in protrumoral cell extrusion

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Poster #11

Epithelia rely on apico–basal polarity cues, changes in adhesiveness and cross-talk with the cytoskeleton network to integrate stimuli and emit coordinated responses that preserve homeostasis. Epithelial polarized cells must remodel their robust and plastic junctions: apical adherens junctions (AJ), which establish the mechanical barrier, and basolateral septate junctions (SJ), which ensure the paracellular barrier. Tricellular junctions (TCJ) integrate mechanochemical and polarity cues at the tissue scale. Three proteins are specifically localized at tricellular septate junctions (tSJ): Anakonda, Gliotactin and M6. Our lab contributed to show how M6 and Aka regulate upstream TCJ assembly by recruiting Gli while other showed M6 synergizes with oncogenic Ras (*RasV12*) to trigger extrusion. I've explored the role of M6 and tSJ to decipher their role in cell extrusion. I've used the larval eye imaginal disc as a model system, a multilayered epithelium composed of an outer squamous cell layer (peripodial epithelium, PE) and an inner pseudostratified layer (disc proper, DP), separated by a lumen and juxtaposed by their apical domains. I found that genetic loss of tSJ synergizes with *RasV12* to induce apical and basal live collective extrusion (ACE and BCE, respectively). Moreover, and in agreement with previous work from our lab reporting an interplay between tri- and bicellular SJ, I found that genetic depletion of a bicellular SJ core component, Nervana-2, in synergy with *RasV12* also induced ACE and BCE. Together, these data show loss of SJ integrity is causal to collective live extrusion. Spatiotemporal reconstruction of ACE and BCE on fixed specimen revealed striking differences in the hierarchy of junctional remodelling, force regulators and adhesiveness during these events. My work provides a comprehensive framework on the role of the paracellular diffusion barrier on tissue integrity and deepends our understanding on the interplay between cell shape, polarity and junctional complexes during collective extrusion.

Keywords: Permeability Barrier, Septate Junctions, Collective Extrusion

*Speaker

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IL-2-mediated induction of regulatory B cells during the early phase of multiple sclerosis

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Flash-talk & Poster #12

Multiple sclerosis (MS) is an autoimmune disease characterized by the infiltration of self-reactive B and T cells into the central nervous system (CNS). It has two main phases : Relapsing-Remitting MS (RRMS), defined by alternating inflammatory attacks and partial recoveries, and Secondary Progressive MS (SPMS), which involves ongoing neural damage.
B cells play a complex role in MS and regulatory B cells (Bregs) have been shown to mitigate disease severity. Various B cells subsets can acquire immunosuppressive capacities after exposure to specific microenvironment signals. Among these signals, we studied the impact of a cytokine called interleukin 2 (IL-2), as we know that IL-2 stimulation could induce Bregs in type I diabetes patients *(Inaba et al., 2023)* and because IL-2 signaling appears disrupted in MS patients *(Maier et al., 2009)*.
In mice, we demonstrated that IL-2 can trigger B cells to secrete IL-10, an immunosuppressive cytokine characteristic of Bregs, after they've been exposed to inflammation mediators, such as IFNg.
In RRMS patients, we observed increased concentrations of IL-2 and IFNg in the cerebrospinal fluid (CSF) compared to controls with other neurological diseases (OND). Overall, the RRMS CSF microenvironment was found in favor of IL-2 signaling on immune cells displaying high-affinity IL-2 receptors. Interestingly, we uncovered a strong correlation between IL-2 and IL-10 in the CSF of RRMS patients but not OND. This suggests that IL-2 signaling in B cells could lead to Bregs polarization and local IL-10 release, possibly explaining the partial remissions observed in RRMS.
Finally, we speculated that B cells from SPMS patients might have reduced responsiveness to IL-2 compared to RRMS, possibly decreasing their IL-10 production and thereby contributing to disease progression. To test our hypothesis, we plan to investigate the activation and IL-2 responsiveness of peripheral B cells from SPMS patients by conducting *in vitro* stimulation experiments.

Keywords: Regulatory B cells, Multiple Sclerosis, IL-2

*Speaker

Induced human pluripotent stem cells to improve diagnosis of SHH-dependent midline brain defects

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Short-talk #3

The identification of causative genetic variants in developmental brain disorders is important for clinical outcome as it allows accurate assessment of prognosis and precise genetic counselling. **Holoprosencephaly (HPE)** is a midline forebrain disease caused by defective developmental processes that take place during early embryonic stages and has a large phenotypic spectrum. Genetics is the major component of this disorder, with alterations leading to a decrease activity of the **Sonic Hedgehog (SHH)** signalling pathway. Our team contributes to improve molecular diagnosis by discovering new variants in the genes involved in this pathology. The impossibility to access the main affected tissues (i.e. the **embryonic anterior neuroepithelium**) is a major obstacle to a pathophysiological study in humans, which is necessary to study the biological impact of these variants. Neuroepithelium modelling using **differentiated iPSC** cells has allowed us to assess the molecular impact of SHH deficiency on human tissue and described new potential genetic markers for HPE. We have established a collection of **transcriptomic signatures** characteristic of the variations of the SHH activity that could be specific for the severity of the HPE phenotype. The relevance of this diagnosis tool will be established using iPSC lines from HPE patients without molecular diagnosis. An RNA signature reminiscent of SHH signalling deficiency will establish that the patient had defective SHH signalling during the early phase of forebrain development and guide further bioinformatics explorations of whole genome data.

Keywords: Sonic, Hedgehog, SHH, Holoprosencephaly, Brain, Genetics, Organoid, Neuroectoderm, Cyclopamine, Stem, Cells, Neurodevelopment, Development, IPSC

*Speaker

Emerging concepts in cancer research and therapy

Choosing unconventional over conventional for therapeutic targeting of protein kinases in cancer: allosteric inhibitors shed light on unexpected pathways and mechanisms of action

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Protein kinases (PK) are hyperactivated in many human cancers thereby constituting relevant biomarkers and attractive pharmacological targets (Fleuren et al. 2016; Wu et al. 2015 ; Roskoski 2021; Cohen et al. 2021). Although a large number of inhibitors targeting the ATP-pocket of PKs have been approved by the FDA, they are often ineffective in monotherapy and suffer limitations, including poor selectivity and emergence of resistance (Wu et al. 2016; Wilson et al. 2018).
Efforts to develop new classes of drugs targeting the conformational plasticity or essential interactions between PKs and their partners are believed to offer promises for more selective therapeutics (Tong & Seeliger 2015 ; Guarnera & Berezovsky 2016; Leroux & Biondi 2020). With the aim of targeting the conformational activation of cyclin-dependent kinases, a class of PKs that coordinate cell growth and proliferation and are notoriously hyperactivated in cancers, we have developed a fluorescent conformational biosensor technology that discriminates against ATP-pocket binding inhibitors, which we have successfully implemented to screen several libraries of small molecules thereby identifying original allosteric inhibitors (Pellerano et al. 2017, Peyressatre et al. 2020).
Lead compounds are new and druggable chemical scaffolds that efficiently inhibit cancer cell proliferation and migration through original mechanisms of action revealing novel and unexplored pathways, which we have investigated through combination of biochemical and imaging approaches in different cancer cell lines. Our studies highlight the relevance of designing smart strategies to engineer conformational biosensors for HTS programmes, as an alternative to conventional activity-based assays, thereby enabling identification of new generations of allosteric inhibitors with promising applications for anticancer therapeutics. By targeting PK plasticity and non-catalytic functions, allosteric inhibitors may further sensitize PKs to more conventional drugs, therefore providing further perspectives for combination therapies by enhancing inhibition of the same target whilst reducing the overall concentration of drugs required for treatment.

Keywords: Kinases, Cancer, Biomarkers

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Identification of molecular actors regulated by IRE1 that control the secretion machinery in glioblastoma cells

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Poster #13

Human glioblastoma (GBM) is the most malignant and aggressive brain tumor, with a high rate of recurrence and mortality. Although the current treatment including surgical resection combined with radiation and chemotherapy has improved, the prognosis for GBM patients is still very poor. Recent studies have shown that IRE1's dual RNase activity through XBP1 mRNA splicing and regulated IRE1 dependent decay (RIDD) of RNA is involved in GBM development and invasiveness. IRE1 is the most conserved sensor of the unfolded protein response (UPR) aiming to resolve endoplasmic reticulum stress, and therefore participates to the control of secreted proteins. In this work, we aimed to investigate whether IRE1 could regulate important molecular actors of the secretory machinery present in GBM cells, that could broadly impact on GBM biology. This study led us to identify different molecules regulated by IRE1 and have an impact on the survival of GBM patients. On-going work is currently conducted to confirm the IRE1-dependent regulation of these candidates in our GBM cellular models focusing primarily on GOLIM4, an integral membrane protein of the cis-Golgi network that plays a role in protein sorting and trafficking within the Golgi apparatus. In addition, the precise mechanism by which these targets impact on IRE1-regulated cell trafficking of secreted proteins will be explored.

Keywords: Glioblastoma, IRE1, Protein secretion machinery, ER stress, UPR

*Speaker

Exploring mitochondrial cristæ as functional and dynamic hubs of the kinase AURKA

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Poster #14

Mitochondria are key organelles in cellular homeostasis, and are often deregulated in cancer. Among the key features of breast cancer conditions, the multifunctional Ser/Thr kinase Aurora A/AURKA is frequently overexpressed. The auto-phosphorylation of AURKA on Thr288 allows the kinase to enter into mitochondria and to localize at the *cristae* and matrix. Overexpression of AURKA causes a deregulation of mitochondrial ATP production, morphology and the degradation of defective organelles by mitophagy.
Our research focuses on the characterization of the interactors of AURKA within mitochondria. Our proteomics-based studies identified that AURKA is interacting with mitochondrial *cristae* proteins. We are studying what impact AURKA overexpression can have on this pool of proteins, and whether by acting on this platform we may counteract the mitochondrial dysfunctions induced by AURKA overexpression. Our previous studies obtained with Förster's Resonance Energy Transfer (FRET) unraveled a strong interaction between AURKA and Prohibitin 2 (PHB2), one of the key *cristae* proteins. We showed that this interaction allows AURKA to orchestrate mitophagy. In AURKA-overexpressing cells, AURKA uses mitophagy to select a pool of mitochondria with high ATP production rates. Mitophagy can be partially rescued using capsaicin, a specific PHB2 inhibitor. Our recent results indicate that the AURKA-PHB2 interaction is strongly reduced in the presence of capsaicin, which also restores mitochondrial mass and morphology.
Our results suggest that AURKA uses *cristae* proteins as a platform to hijack mitochondrial functionality and to sustain cell proliferation. They also pave the way to the targeting of the AURKA-PHB2 interaction as a pharmacological strategy to revert the consequences of AURKA overexpression in cancer conditions.

Keywords: Mitochondria, Cancer, AURKA, Microscopy, Protein protein interaction

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Canine oral melanomas: immunocompetent models for the genetics and therapy of mucosal melanomas in humans

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Poster #15

Mucosal melanomas (MM) are a rare subtype of human melanoma: usually located in the oral, nasopharyngeal or ano-vaginal spheres, they are extremely aggressive, with a 5-year survival rate of just 20-25%. These MM present a distinct pathophysiology from cutaneous melanomas, and are without effective therapy to date.

Dogs naturally develop MM (mainly oral): certain canine breeds are predisposed, and they present very similar clinical, histological and treatment-response characteristics to humans. The aim of the project is to characterize these canine tumors, in order to develop natural models of human MM. We have thus developed a dozen primary canine cell cultures directly from tumor exudates, and characterized them in terms of their tumorigenicity in vivo in mice, their metastatic potential through the development of bioluminescent lines, and their genetic potential through their sequencing ("Low pass" : 1X coverage).

These cell cultures were used to evaluate the effects of ten or so drugs on cell proliferation, as well as their tumorigenicity and metastatic potential in immunodeficient mice; their sequencing enabled us to pinpoint the mutated oncogenic pathways of each lineage.

We now have a panel of 12 canine primary cultures, each with its own genetic characteristics, enabling us to screen therapies targeting human MM (new drugs and repositioning) for new predictive biomarkers. This preclinical, spontaneous and ethical model (samples taken with the owner's permission, in the dog's care pathway) will enable the discovery of new therapeutic targets and better stratification of patients for the benefit of veterinary and human medicine.

Keywords: Mucosal melanoma, Oral melanoma, Canine tumors, Immunocompetent model, Oncogene pathways, Genetic characterization, Therapeutic targets, Dog, Human

*Speaker

The HDAC inhibitor belinostat sensitizes ovarian cancer tumoroids to the PARP inhibitor olaparib

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Flash-talk & Poster #16

The recent introduction of PARP inhibitors (PARPi), such as olaparib, into treatment protocols for ovarian cancer has been a major advance for this pathology with a poor prognosis. Inhibition of PARP by these molecules leads to the accumulation of DNA double-strand breaks, which are normally repaired by the homologous recombination (HR) pathway. When this HR is deficient, cells are unable to repair PARPi-induced damages and die by a mechanism of "synthetic lethality". PARPi are therefore indicated for HR-deficient tumours. However, in more than half of the patients, tumours display a functional HR pathway and do not respond to this targeted therapy. Identifying PARPi sensitization strategies constitutes a major challenge for these patients.

HDAC inhibitors (HDACi), which modulate gene expression, have been described as capable of disrupting the HR pathway by indirectly silencing some of its effectors. In this context, our objective was to evaluate the interest of belinostat, a pan-HDAC inhibitor used in the clinic, to sensitize ovarian cancer cells to olaparib. In this work, we investigated the efficacy of the belinostat/olaparib combination on both the SKOV3 ovarian cancer cell line and on different three-dimensional tumoroid models, derived from patients' tumours and representative of them, using various techniques assessing cell viability and apoptosis.

We first showed that belinostat efficiently sensitized the SKOV3 cell line to olaparib. We then validated this result in more than half of our tumoroid models, in which the belinostat/olaparib combination highly decreased viability in contrast to each molecule used alone. Moreover, our results suggested that this sensitizing effect could be ascribed to apoptosis induction. Interestingly, the tumoroids that were sensitive to the combination displayed more severe DNA damage than the resistant ones.

These data thus suggest the interest of using HDACi to sensitize ovarian cancer to PARPi, with predictive markers for response still to be determined.

Keywords: Ovarian cancer, HDAC inhibitor, PARP inhibitor, Sensitization strategies, Tumoroid

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Regulation of death receptor signalling by UFMylation in triple-negative breast cancer cells

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Poster #17

Signalling induced by the death receptors CD95 and TRAIL-R1/R2 has been described to regulate tumour progression. Ligands of these receptors (CD95L and TRAIL, respectively) can induce cytotoxic pathways leading to the death of target cells. However, DRs can also induce proliferation, cell migration and cytokine production by activating signalling pathways such as NF-κB and MAPKs. These are the non-cytotoxic pathways of DRs. The induction of these different pathways by DRs is based on the formation of a key protein complex, DISC (DISC for Death Inducing Signalling Complex) associated to TRAILR/2 or CD95 upon stimulation by their cognate ligands. Following activation by their respective ligand, CD95 and TRAIL-R1/R1 oligomerise and then recruit different proteins in a cascade to form DISC. Signalling induced by CD95L and TRAIL is tightly regulated, mainly by post-translational modifications (PTMs). Preliminary data from the laboratory indicate that UFL1, the E3 ligase that catalyses the transfer of a ubiquitin-like PTM (UFMylation), may control apoptosis induced by TRAIL and CD95L. I demonstrated that UFL1 decreases the expression of DISC constituents, and represses the formation of this protein complex essential for DR signalling. Thus, UFL1 decreases the activation of apoptotic signalling by CD95L and TRAIL. With regard to non-cytotoxic pathways, UFL1 reduces activation of the NF-κB, p38 and JNK pathways and conversely promotes activation of the ERK pathway. Taken together, our results reveal that UFL1 is an early regulator of apoptotic and non-cytotoxic signalling mediated by the DR CD95 and TRAIL-R1/R2.

Keywords: DR, CD95, TRAILR1, TRAILR2, DISC, UFMylation, UFL1, MAPK, NF-κB, Caspase8, Apoptosis

*Speaker

Inhibition of SERCA2 calcium pumps sensitises chemoresistant ovarian carcinoma cells to the BH3-mimetic ABT-737

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Flash-talk & Poster #18

Ovarian cancer has a poor clinical prognosis due to innate or acquired chemoresistance following carboplatin/paclitaxel treatment. PARP inhibitors have revolutionised patient management, but they are only offered to patients with Homologous Recombination Deficiency who have responded to carboplatin treatment. There are therefore no effective options for patients who have not responded to chemotherapy. The chemoresistance can be explained by an unbalanced ratio of anti-apoptotic (Bcl-xL, Mcl-1) to pro-apoptotic (Bim, Puma, Noxa) Bcl-2 family members that prevents cell apoptosis. In this context, the BH3-mimetic molecule, ABT-737, which targets Bcl-xL, triggers apoptosis when it's combined with Mcl-1 inhibitors or pro-apoptotic inducers.

Calcium signaling deregulation is strongly involved in carcinogenesis and the calcium pumps SERCA2 that fill the ER with calcium are implicated in cancer cell fate. Their inhibition induces pro-apoptotic members expression in some models through ER stress activation. Therefore, we evaluated the interest of SERCA2 pumps inhibition to sensitize chemoresistant ovarian cancer cells to ABT-737.

The platinum-resistant cell line, OAW42-R, was treated with anti-SERCA2 strategies (siRNA or thapsigargin), in combination with ABT-737. These co-treatments induced a strong Noxa- and caspase-dependent apoptosis that involved UPR response and more precisely ATF4 transcription factor activation. Patient-derived ovarian cancer organoids, an innovative three-dimensional model designed to predict clinical responses, were also exposed to thapsigargin/ABT-737 treatment, which induced a massive destructuration of the organoids.

Our study shows for the first time that targeting SERCA2 calcium pump and inducing UPR response could be a relevant strategy in ovarian cancer treatment. The ER stress inducer ONC201, used in clinical trials for various cancer treatments, confirms the value of targeting ER stress in cancer therapy. Moreover, the use of mipsagargin, a pro-drug of thapsigargin in phase II clinical trials in prostate cancer treatment consolidates the therapeutic interest of our study and offers new hopes for patient management.

Keywords: Ovarian cancer, Chemoresistance, Apoptosis, Bcl-2 family members, Calcic signalisation, SERCA, Tumoroids.

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The role of DIS3-mediated RNA surveillance in the physiopathology of Multiple Myeloma

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Flash-talk & Poster #19

Multiple Myeloma (MM) is a cancer of plasma cells and is still incurable to date. A better understanding of the molecular mechanisms of the disease should provide new strategies for diagnosis and therapy that are critically needed to improve the patients' lives.
The *DIS3* gene is frequently and specifically mutated in MM. In mice, *DIS3* knockout significantly alters B cell development at multiple stages, especially during the genetic recombination events that punctuate the differentiation process. This gene encodes the catalytic subunit of the ribonuclease RNA exosome complex that contributes to the degradation of various categories of coding and noncoding RNAs. DIS3-deficient B cells accumulate noncoding transcripts that are suspected to disturb both transcription and 3D genome structure, thus impairing the differentiation process. The mechanistic studies on the role of DIS3 in B and plasma cell differentiation were all performed in mouse models but never in humans.
This research project thus aims to decipher the role of DIS3-mediated RNA surveillance during plasma cell differentiation and to understand how alterations of this process can relate to MM. We use an optimised *in vitro* differentiation model that allows us to reproduce plasma cell differentiation starting from human naïve B cells purified from healthy donor buffy coats. The cells are differentiated either in native conditions or in the presence of vivo-morpholinos targeting the *DIS3* transcripts that mimic a loss of function of the protein. We can then investigate the consequences on the phenotype (FCM analysis for surface markers), the coding and noncoding transcriptome (deep RNAseq), genome instability (chromosome instability, translocations), the 3D genome structure (3C), cell cycle and mitosis... We thus hope to provide a sharper understanding of how *DIS3* loss of function contributes to MM pathogenesis, and to propose a potential target for future therapies.

Keywords: Multiple myeloma, DIS3, RNA surveillance, B cells, Plasma cells

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Gene expression profiling of peripheral blood mononuclear cells from women with cervical lesions reveals new markers of cancer

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Short-talk #4

Cervical cancer (CC) is a multifactorial disease of which human papillomavirus (HPV) is the main etiological agent. Despite cervical Pap smear screening and anti-HPV vaccination, CC remains a major public health issue. Identification of specific gene expression signatures in the blood could allow better insight into the immune response of CC and could provide valuable information for the development of novel biomarkers. The present study performed a transcriptomic analysis of peripheral blood mononuclear cells (PBMCs) from Senegalese patients with CC (n=31), low-grade cervical intraepithelial neoplasia (CIN1; n=27) and from healthy control (CTR) subjects (n=29). Individuals in the CIN1 and CTR groups exhibited similar patterns in gene expression. A total of 182 genes were revealed to be differentially expressed in patients with CC compared with individuals in the CIN1 and CTR groups. The IL1R2, IL18R1, MMP9 and FKBP5 genes were the most upregulated, whereas the T-cell receptor α gene TRA was the most downregulated in the CC group compared with in the CIN1 and CTR groups. The pathway enrichment analysis of the differentially expressed genes revealed pathways directly and indirectly linked to inflammation. To the best of our knowledge, the present study is the first large transcriptomic study on CC performed using PBMCs from African women; the results revealed the involvement of genes and pathways related to inflammation, most notably the IL-1 pathway, and the involvement of downregulation of the T-cell receptor α, a key component of the immune response. Several of the stated genes have already been reported in other cancer studies as putative blood biomarkers, thus reinforcing the requirement for deeper investigation. These findings may aid in the development of innovative clinical biomarkers for CC prevention and should be further replicated in other populations.

Keywords: Cervical cancer, Cervical intraepithelial neoplasia, Peripheral blood mononuclear cells, Blood biomarker, Transcriptomics

*Speaker

Development and biological evaluation of PROTAC molecules directed against Mcl-1 and/or Bcl-xL for the treatment of ovarian cancer

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Poster #20

The development of new therapeutic strategies is a major challenge for improving the management of ovarian cancers, which are among the most deadly in women. The anti-apoptotic proteins Mcl-1 and Bcl-xL, whose expression is strongly correlated with resistance to treatment, are relevant targets, but the development of pharmacological inhibitors of these proteins is currently hampered by associated toxicities (thrombocytopenia linked to inhibition of Bcl-xL, cardiac toxicity for inhibition of Mcl-1). PROTAC (PROteolysis TArgeting Chimera) technology, which induces targeted degradation of proteins by the ubiquitin-proteasome system, could be used appropriately in this context. This strategy involves combining a ligand specific to the target protein with a ligand targeting an E3 ubiquitin ligase, absent from the tissues to be avoided but present in the targeted tumour. We have previously demonstrated that one of the original molecules we have developed, Pyridoclax, exhibits Mcl-1 inhibitory activity. On the basis of this work, we have developed and evaluated the activity of PROTAC molecules based on the coupling of Pyridoclax or its derivatives to ligands targeting the E3 ubiquitin ligases VHL and CRBN (little or not expressed in platelets and heart but expressed in ovarian cancers). Their effect on the expression of Bcl-xL and Mcl-1 proteins was assessed by western blot, showing that some of them are active (up to 90% extinction) at nanomolar concentrations. Their effect on the viability of ovarian tumour cells, alone or in combination with various anti-cancer agents, is currently being evaluated.

Keywords: Ovarian cancers, Apoptosis, Bcl-2 family, PROTAC molecules, Protein degradation

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Establishment of tumor organoids from head and neck squamous cell carcinoma and evaluation of their response to conventional and innovative therapies

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Short-talk #5

Introduction: The risk of relapse or recurrence of head and neck squamous cell carcinoma (HNSCC) is high despite the combination of surgery and radiochemotherapy, responsible for high toxicity. It is crucial to develop new therapeutic strategies and to identify patients likely to benefit from these treatments. Patient-Derived Tumor Organoids (PDTO) are three-dimensional multicellular structures obtained from patient tumor samples and faithfully reproduce the histological and molecular characteristics of the original tumor. A growing body of research indicates that PDTO may predict the clinical response, representing a major opportunity for the development of new therapeutic strategies and precision medicine.
MetM: PDTO were obtained after dissociation of tumor specimen of HNSCC patients thanks to the setup of the clinical study ORGAVADS (NCT04261192). Tumor cells were embedded in extracellular matrix and cultured in specific medium. Histological and immunohistochemical characterizations were performed to validate the resemblance between PDTO and their original tumor. Response of PDTO to chemotherapy, radiotherapy and innovative therapies were evaluated by live-cell imaging and viability assay.
Results: The culture conditions were optimized to improve the success rate of PDTO establishment which now exceeded 50%. Twenty-one PDTO have been established and showed histological and immunohistochemical characteristics close to the original tumor. Functional assays were performed on 15 PDTO to analyze the response to treatments and heterogeneity of response was observed between the PDTO. Two PDTO derived from HPV+ oropharyngeal tumor displayed very high sensitivity to cisplatin, matching with the patient's profile and response. When clinical response was available, PDTO derived from responders showed high sensitivity to cisplatin or X-rays while PDTO from non-responders were resistant to these treatments.
Conclusion: These first results showed feasibility to obtain PDTO from HNSCC and perform functional assay to assess their response to different treatments. First comparisons between response of PDTO and clinical response are promising.

Keywords: Tumor organoids, Predictive medicine, HNSCC

*Speaker

Identification of circRNAs involved in acquisition of BRAFi resistance in metastatic melanoma

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Poster #21

Fifty percent of the patients diagnosed with metastatic melanoma harbor the driver mutation V600E of the kinase BRAF. BRAF inhibitors (BRAFi) combined with MEK inhibitors (MEKi) are the main treatment in this case. However the majority of patients develop resistance.
It is now well established that the microenvironment, intrinsic cell factors and the drug pressure, participate to the switch from a proliferative to invasive state in melanoma cells, acquiring resistance to targeted therapies.
The Goal of this study is to explore the hypothesis that this plasticity can be driven by a variety of noncoding RNA : circular RNA. CircRNAs are highly represented in the eukaryotic transcriptome and can function as a super miRNA sponges to prevent their degradation function on target mRNAs.
First using global bioinformatics approaches, we aimed to decipher the role of the circRNA-miR-mRNA network in the regulation of BRAFi resistance genes in melanoma. Then, we particularly focused on the regulation of the expression of critical regulators of resistance such as AhR and AXL. Using luciferase reporter assay, we identified specific miR-MRE interactions onto AhR and AXL 3'UTR. In parallele, overexpression of respective miRs after transfection of synthetic miRs (mimics) led to the specific downregulation of AhR and AXL expression in SKMel28 cells.
Furthermore, we showed that the depletion of the specific circRNAs circ_1610 by siRNAs, led to the decrease of the expression of both AhR and AXL after the release of endogenous miRs (miR-29a-3p, miR-151a-3p).
In addition, to decipher the role of identified miRNAs and circRNAs for the control of BRAFi-resistance and melanoma plasticity, we established SKMel-28 stable cell lines, invalidated for the different circRNAs (CRISPR- Cas13) or that overexpressed constitutively the miRNAs.
Finally this study will open new therapeutic strategies of melanoma using specific antisens oligonucleotides (ASO). Indeed, we plan to specifically target circRNAs to counteract BRAFi resistance.

Keywords: Melanoma, BRAFi resistance, Non coding RNA, CircRNA, MiRNA

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Use of patient-derived tumor organoids to assess ovarian cancer response to chemotherapy and PARP inhibitors

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Poster #22

Ovarian cancers are the leading cause of death from gynecological cancers in France, due to a late diagnosis combined with the development of resistance to chemotherapy. However, half of these cancers present alterations in homologous recombination (HR), making them sensitive to inhibitors of the PARP protein (PARPi), involved in DNA repair. Nevertheless, identifying patients who respond to chemotherapy and selecting those eligible for PARPi remains a challenge for clinicians. In this context, the use of patient-derived tumor organoids (PDTO) for predictive functional testing represents an interesting prospect. The aim is to study the feasibility of a PDTO-based predictive strategy in order to evaluate its potential clinical application.

30 PDTO models were generated from 24 ovarian cancer patients. Through histological and immunohistochemical analyses (study of Ki67, PAX8 and p53), we were able to show that PDTO models reflect the histological characteristics of their original tumor. In parallel, global approaches (CNV and transcriptomic profiling) were realized to study the relevance of the tumor organoid model in molecular terms in relation to the original tumor.

To demonstrate the relevance of PDTO as a predictive tool, we exposed 23 different PDTO models from 21 patients to conventional therapies (carboplatin, paclitaxel and PARP inhibitors). The correlation of PDTO sensitivity to carboplatin and the patient's clinical response is currently underway. Otherwise, we are working on correlating the sensitivity of PDTO to PARPi with a functional test studying the ability of PDTO to initiate HR (RECAP test).

Finally, in order to get closer to the conditions required for clinical application there is a need to accelerate functional test results and to adapt our protocols. To this end, PDTO sensitivity in 384-well plates has been developed, enabling us to obtain the same results as conventional processing in 96-well plates, with 4 times less biological material.

Keywords: Patient, Derived tumor organoids, Ovarian cancer, Predictive medicine, Functional testing, Drug screening

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Advanced methods for the study of biological phenomena

Optical and computational lenses into biological processes

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Advances in microscopy and artificial intelligence (AI) are fueling progress across the life sciences. Our lab develops imaging and modeling techniques to quantitatively describe and, where possible, predict cellular organization or dynamics. We then apply these techniques to address specific biological questions, often in collaborations. The first part of this talk will highlight how we combine optics and algorithms to push the limits of single molecule super-resolution microscopy along spatial, temporal and spectral dimensions in order to better characterize biological structures and their dynamics. The second part will showcase two recent or ongoing biology-driven projects. It will illustrate how we combine super-resolution imaging and physics-based modeling to quantitatively characterize cohesin-dependent chromatin loops, key drivers of chromatin organization and function. I will also show how we co-develop a pipeline using high-throughput imaging and AI to characterize drug-induced phenotypes in bacteria. Preliminary results indicate that the mechanisms of actions of drugs may be predictable from imaging data alone, paving the way for a pipeline to accelerate antibiotic drug discovery.

Keywords: AI, Bioinformatics, Microscopy

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Microtubule structural instability

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Poster #23

Microtubules are polymers of tubulin that form part of the cytoskeleton and provide structure and shape to eukaryotic cells. Microtubules can be as long as 50 micrometres, as wide as 23 to 27 nm. They are formed by the polymerization of a dimer of two globular proteins, alpha and beta tubulin into protofilaments that can then associate laterally to form a hollow tube, the microtubule. Usually, microtubules are depicted as almost perfect polymers of the tubulin heterodimer that shares homotypic interactions between its $\alpha\beta$-subunits, except at a unique region called the 'seam'. To address this paradigm, we decorated microtubules with kinesin motor domains, performed 3D reconstructions by dual-axis cryo-electron tomography, and developed a segmented sub-tomogram averaging strategy to address their structural heterogeneity. We found that when assembled from purified tubulin, microtubules can contain several seams whose number and location vary along their length, leaving holes within their shaft. Those assembled in Xenopus egg cytoplasmic extracts are much more homogeneous, but still incorporate variations in seam number and location, suggesting a tightly regulated process in a cytoplasmic environment. These observations prompted us to propose the concept of 'microtubule structural instability' that may be involved in their particular dynamic behavior known as 'microtubule dynamic instability'. We anticipate that microtubules associated proteins such as end-binding proteins could exploit this unique structural property to finely tune their dynamics in cells.

Keywords: Microtubule, Electron microscopy, Structure

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Delivery of AuNCs into extracellular vesicles by fusion using ionizable liposomes cargos

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Short-talk #6

Extracellular vesicles (EVs) are macromolecule carriers involved in cellular communication, produced from non-apoptotic cells. They can be exploited for high-tech medicine delivery, but efficient loading with therapeutic or imaging agents remains an obstacle.
Au NCs are biocompatible, non-toxic fluorophores used for biological labeling, bioimaging, and biosensing due to their brightness and photoluminescence duration, furthermore their ultra small size make them easier to clear through the kidney pathway.
This study aims to analyze EVs' content using luminescent nanoparticles, focusing on the correlation between lumen content and membrane composition, which could provide insights into the origin and functions of EVs, potentially aiding in understanding biological mechanisms..
For this, AuNCs, thanks to their size and properties, represents the best candidate to understand the nature of different EVs and explore their possible application as highly bio-compatible delivery system. The small size of luminescent NCs allows for the encapsulation of them into liposomes without compromising compartment integrity, allowing them to be delivered into extracellular vesicles.
We have explored the possible encapsulation of ultra-small-sized red and blue emitting Au NCs into liposomes of various sizes and chemical compositions. We confirmed the possible encapsulation and the efficiency of the process was correlated to the structural and morphological aspect via complementary analyses by SAXS, cryo-TEM, and confocal microscopy techniques. Finally, exosome-like-sized vesicles (LUVs) containing Au NCs were obtained with an encapsulation yield of 40%, as estimated from ICP-MS.
Briefly, this study explores the interaction between fusogenic liposomes encapsulating AuNCs and EVs from human seminal fluids. The membrane composition was optimized for better fusion properties. The mixture results in lipid exchange and increased size, exhibiting AuNCs fluorescence and in increased size revealed by flux cytometry. The resulting fused hybridosomes were isolated using SEC, displaying the typical AuNCs fluorescence and increased size in line with expected fused exosome size.

Keywords: Cellular communication, Gold nanoclusters, Extra, Cellular vesicles, Early diagnosis, Encapsulation, Delivery systems

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Peptidic luminescent Gold nanocluster for biosensing

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Short-talk #7

Gold nanoclusters (AuNCs) appear as a recent class of non-toxic fluorophores. Their brightness, their ultrasmall size ($<$ 2 nm) and large window of fluorescence lifetime (1ns – 1ms) and their good biocompatibility make them an attractive alternative as fluorescent probes for biological labeling and bioimaging.

We demonstrated their in vivo targeting ability because of specific peptidic recognition groups (R. Cheichio et al. ACS Nano materials **2023**)

Here we present the direct synthesis of original ultrasmall luminescent peptidic gold nanoclusters sensitive to pH. The versatility of small peptides permits to adjust the emission wavelength and the sensitivity of the emission intensity as well as the luminescent lifetime. This chemical plateform provides AuNC dual nanoprobes with potential sensitivity to the environment for biosensing applications. Such nanostructures offer promising candidates for fluorescent in vivo biosensing and biolabeling.
We also investigated the interaction of AuNC with Extracellular vesicles (EVs) which are well-known membrane-limited particles that are secreted by healthy and cancerous cells. EVs are identified in human follicular fluid as a mode of communication in the ovarian follicle (Neyroud A. S. et al. Int. J. Molecular Sci. **2022**). In addition EVs involved in cell-cell communication are considered as biomarkers for early cancer diagnosis. The analysis of their content thanks to AuNCs nanoprobes could provide informations about the origin and the biological function of the EVs. The EVs labeling with easily detectable nanoparticles could enable the development of a powerful tool for the early diagnosis of specific diseases.

Keywords: Peptides, Gold nanocluster, Biosensing, pH sensing

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Cell cycle phases classification based on object detection models

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Poster #24

Current optical microscopes are now fully motorised, calling for making them smart and automatically adjusting the acquisition modalities to objects and events detected in the image. Especially in high content screening, the image classification is either performed through customised software, requiring re-development for each biological case, or posteriorly entailing fastidious and sample-damaging acquiring of every object. Nowadays, CNNs outperform all existing techniques in classifying cell images. In our previous work, we proposed a semi-supervised deep-learning based on a GAN for cell cycle phase detection to support autonomous microscopy. This method requires a segmentation before classifying the images by SGAN. Recent deep learning methods, called object detection methods, can locate cells in images and classify them using only one neural network. We tested it using images of cells during the cell cycle, especially mitosis phases, namely Interphase, Prophase, Prometaphase, Metaphase, Anaphase, Telophase and Junk; this last class containing dead cells or non-cellular objects. We identified three specific challenges: (1) we can only access a reduced set of training, here 30 real-images, and the set is strongly unbalanced; (2) objects are small and numerous on the image in contrast with the classic cases in object detection (Crowded scene problem); (3) objects can frequently touch each other's. We benchmarked three algorithms: Faster RCNN, RetinaNet and Yolo; all were pre-trained using the Coco dataset. We then performed a full training of this pre-trained network. To balance the classes, we created 600 semi-synthetic microscopy images by assembling vignettes of individual cells extracted from the 20 real images of the training set. Tested on the 300 semi-synthetic images from the remaining 10 real images, Yolo and RetinaNet yielding F1- measure 95.5% and 88%, respectively, while classifying a 416x416 pixels image in 0.01 and 0.02 seconds. Preliminary experiments on real images suggest that YoLo is more accurate.

Keywords: Object Detection, Deep Learning, Cell cycle phases, Neural Network

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Expansion microscopy of Xenopus egg extract spindles

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Poster #25

The spindle is a bipolar structure, made of up to hundreds of thousands of microtubules (MTs), which can adapt its size and architecture among cell types and organisms to ensure the faithful segregation of chromosomes. Although many aspects of spindle assembly have been depicted through the past decades, the precise mechanisms by which the spindle assembles and regulates its morphology remains unclear. To address this question, we use *X. laevis* and *X. tropicalis* egg extracts. Indeed, *X. tropicalis* spindles are not only shorter than their *X. laevis* counterparts, but they are also architecturally different. To study this complex structure, we set out to adapt expansion microscopy, a method by which the resolution increases by physically enlarging the sample by embedding it in a swellable gel, while remaining observable with conventional microscopes. As this method has not yet been applied to *Xenopus* egg extracts, we developed an optimized protocol depending on our sample characteristics. We thus compared different conditions by measuring their expansion factor, the conservation of the spindle architecture, and possible deformations due to expansion. We are now able to analyze the microtubule organization and the distribution of molecular motors, and compare them between the two *Xenopus* species with unprecedented details.

Keywords: Cell division, Spindle, Microtubules, Expansion microscopy, Xenopus egg extracts

*Speaker

Impact of ADP-ribosylation on PARP1 dynamics at DNA level.

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Poster #26

Poly-ADP-ribose polymerase 1 (PARP1) is a protein that initiates the response to DNA damage. Its role is to detect DNA lesions, bind to them, and then initiate the repair process by adding ADP-ribose chains to itself and histones near the damaged sites. In case of inhibition of its catalytic activity using small-molecule inhibitors, PARP1 is trapped on damage, which blocks the repair process. PARP inhibitors are lethal for BRCA-mutated cancer cells due to their sensitivity to this trapping phenomenon. This underscores the fundamental importance of comprehending the mechanisms governing PARP1-DNA interactions for therapeutic purposes.

Our project aims to explore the impact of ADP-ribose chains on PARP1 dynamics at DNA level independently of the presence of DNA lesions. Our first step was to create experimental conditions where ADP-ribose accumulates in the nuclei of living cells. We obtained this condition by impairing the activity of two erasers of ADP-ribose chains: ARH3 (knock-out) and PARG (inhibitors).Then we used Fluorescence Correlation Spectroscopy (FCS), a microscopy technique giving the possibility to compare the residence time of GFP tagged PARP1 in the confocal volume by analyzing fluorescence fluctuations arizing from protein motions. We used FCS to compare PARP1 dynamics between control cells and cells showing increased ADP-ribose signaling.

In conclusion, we observed a reduced residence time of PARP1 in nuclei with an accumulation of ADP-ribose chains, indicating heightened PARP1 dynamics at the DNA level. These findings suggest that ADP-ribose chains reduce the affinity between PARP1 and DNA, consequently impeding the dynamic association between PARP1 and the DNA. Taken together our results indicate that this post-translational transformation is involved in the dissociation of PARP1 from DNA lesions.

Keywords: DNA repair, Cancer, Fluorescence correlation spectroscopy

*Speaker

Characterizing the interplay between neddylation and isomerization in the Ubiquitin-Proteasome System regulation

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Poster #27

The Merkel cell carcinoma (MCC) is a highly aggressive cancer with a low prognosis, and it is rare, with an incidence of 2,500 cases per year in Europe. The Merkel cell polyomavirus (MCPyV), discovered in 2008, is responsible for 80% of the cases of transformation. This virus integrates into the host cell's genome. The virus codes for six proteins, of which only two are expressed in MCCs and have oncogenic roles: LT (large T antigen) and sT (small T antigen). LT is expressed in a truncated form, having lost the part responsible for virus replication. This minimal transformation system allows for a systematic study of the associated oncogenic mechanisms. The project's aim is to identify the transforming mechanisms induced by MCPyV and, more broadly, new oncogenic pathways. Using proximity-based interactomics techniques (BioID and SplitID), we have been able to identify critical interactions between oncoviral proteins and host proteins. Our preliminary results have already highlighted a potential oncogenic axis involving LT-PIN1-NEDD8-SCF/C (SKP1-Cullin-FBox ubiquitin ligase complex). PIN1 is a cis-trans proline isomerase that recognizes phospho-serine/threonine motifs in proteins. It is implicated in numerous neurodegenerative and cardiovascular diseases and is associated with poor prognosis in several types of cancer. Through NEDD8, a post-translational modification in the ubiquitin-like (UBL) class, PIN1 appears to be targeted to SCF/C and may regulate its activity. We identified this PIN1-NEDD8 interaction in close proximity to SCF/C components. Furthermore, we have shown that PIN1 is not a substrate of NEDD8, unlike Cullins. Our study aims to identify an innovative mechanism of regulation of the ubiquitin system that is diverted by oncogenesis in our MCC model.

Keywords: Merkel cell carcinoma, Ubiquitination, Neddylation, Isomerization, Interactomics

*Speaker

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Identification of key players in IRE1 signaling through interactome analysis

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Poster #28

The unfolded protein response (UPR) is a key mechanism that primarily aims to restore endoplasmic reticulum (ER) homeostasis and is likely involved in other adaptive pathways of particular relevance to cancer. The UPR is transduced by three proteins acting as sensors and triggering signaling pathways. Among them, IRE1α, an ER-resident type I transmembrane protein, exerts its function through both kinase and endoribonuclease activities that result in both XBP1 splicing and RNA cleavage (Regulated IRE1 Dependent Decay - RIDD) through mechanisms that remain to be clearly documented. Recently an increasing number of studies have reported that protein-protein interactions (PPi) regulate IRE1α signaling, and can drive additional non-canonical functions associated with this protein. Here, we developed a BioID-based approach in HEK293T cells aiming to label proteins in proximity of IRE1α in situ. The resulting IRE1α interactome identified 206 proteins, of which 25% appear during ER stress. We further define the IRE1α signalosome (i.e. proteins in complex with IRE1α that affect signaling properties of a protein) based on literature, computational analyses and subsequent experiments, allowing us to document how PPi may regulate the IRE1α pathway and the subsequent biological outcomes. Moreover, we hypothesize on potentially novel IRE1α functions based on the nature of its interactome and its localization.

Keywords: IRE1, Endoplasmic reticulum, UPR, BioID

*Speaker

Nextflow pipelines for Genomic Imputation: From Phasing to Imputation to Validation

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Short-talk #8

In the realm of genetic research, the utilization of low-pass sequencing techniques introduces a critical challenge: the accurate reconstruction of haplotypes and the imputation of missing genetic data generated by this new sequencing technology. This challenge becomes particularly pronounced when dealing with species lacking dedicated phased panels, as observed in canids and felids. Proper haplotype reconstruction serves as the foundational step toward constructing a reliable reference for subsequent imputation, thereby enhancing the comprehensiveness of genetic information.

Addressing this challenge demands a systematic approach. We present an nf-core-compliant pipeline meticulously devised for haplotype phasing, imputation, and validation. This tool amalgamates cutting-edge phasing and imputation methodologies, empowering researchers to harness the full potential of their genetic data. This endeavor not only bridges genetic information gaps but also harmonizes disparate datasets and augments the precision of genetic analyses.

By adhering to nf-core standards, our pipeline guarantees a robust analytical framework, incorporating up-to-date tools, version control, rigorous testing, and a supportive community. This, in turn, offers a profound advantage to researchers operating within the domain of FAIR (Findable, Accessible, Interoperable, and Reusable) principles.

In essence, our dedicated nf-core pipeline unveils a comprehensive solution for efficient genomic imputation, encompasing vital stages of haplotype phasing and validation. Its deployment significantly streamlines research efforts, ensuring researchers derive maximum insights from their genetic datasets. These benefits are not limited by species boundaries, extending the utility of the pipeline to diverse organisms. Importantly, by adhering to FAIR principles, our tool furnishes standardized, reproducible, and collaboratively-endorsed avenues for addressing genomic imputation challenges.

Keywords: Bioinformatics, Imputation, FAIR

*Speaker

Phenotyping mouse tissue myeloid and lymphoid cells by mass cytometry

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Flash-talk & Poster #29

Immune cells have a key role in maintaining tissue homeostasis. Genetic and environmental factors may induce changes in their number and functions that can be responsible for the development of organ pathologies and diseases. The study of immune cell populations requires the development of more powerful single-cell techniques, enabling us to study the complexity of these cells in pathological contexts. Mass cytometry combines the principles of flow cytometry and mass spectrometry and allows precise single-cell phenotyping and quantification of numerous cell sub-populations simultaneously. This method uses until 40 metal-coupled antibody panel to detect surface and intracellular cell markers. Our aim was to develop a mass cytometry antibody panel allowing the identification of lymphoid and myeloid cells isolated from different tissues and fluids from healthy and non-healthy mice.

A 37 antibody panel was designed to identify macrophages, dendritic cells, granulocytes and lymphocyte sub-populations. Some antibodies unavailable required to be custom-coupled to the metal. Antibodies were titrate using a cocktail of different cell types to evaluate the optimal concentration to use. Tissue dissociation protocols were optimized to improve cell yield and viability and the effects of tissue thawing were studied. Cell suspension obtained were stained with 30 surface antibodies and 7 intracellular antibodies. Cells were next acquired on the Helios mass cytometer at the Hyperion platform of Brest.

This methodology has been validated on lung, spleen and intraperitoneal lavage cells, making it possible to distinguish between different sub-populations of immune cells in healthy and pathological contexts. This protocol allows the phenotyping of myeloid and lymphoid cells in fresh lung but not in thawed lung samples due to a lower cell viability. However, it is applicable to thawed spleen and to thaw cells from intraperitoneal lavages with unfortunately some epitopes altered.

*Speaker

Deep learning for predicting the response to nivolumab in metastatic clear cell renal cell carcinoma

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Poster #30

The Nivoren study was based on a cohort of metastatic clear cell renal carcinoma patients from multiple centers. The goal was to study Nivolumab response after failure of anti-angiogenic treatment (tyrosine kinase inhibitors). Whole-slide images (WSIs) stained with Hematoxylin-Eosin-Saffron were processed with deep learning with the assumption that there was an unknown visual phenotype able to predict Nivolumab response.

WSIs are too large to be processed at once by a computer. Therefore, the following procedure was implemented: first, every slide was tiled in many patches, at varying magnifications. Then, these patches were encoded by a neural network (ResNet50 pretrained on ImageNet) in order to extract meaningful features. Finally, these encoded patches were used to predict the response to Nivolumab, with another neural network. To train and evaluate this pipeline, response to Nivolumab was evaluated based on two criteria: the best overall response which indicates how well the patient responded to treatment, and the progression free survival which is the number of months before relapse. Other criteria such as prediction of cancer grade, vascular endothelial growth factor (VEGF) and tertiary lymphoid structure (TLS) presence were also tested. For each criterion, a different model was trained to associate to the image the corresponding label.

Predicting response to Nivolumab in this context was not conclusive. It is likely that either there is no visual phenotype or the amount of data was insufficient. However, prediction of other criteria, often linked to more visible phenotypes such as grade, VEGF levels or TLS presence is possible, achieving AUC $>$ 0.75.

Keywords: Deep learning, Convolutional neural networks, Renal cancer, Pathology

*Speaker

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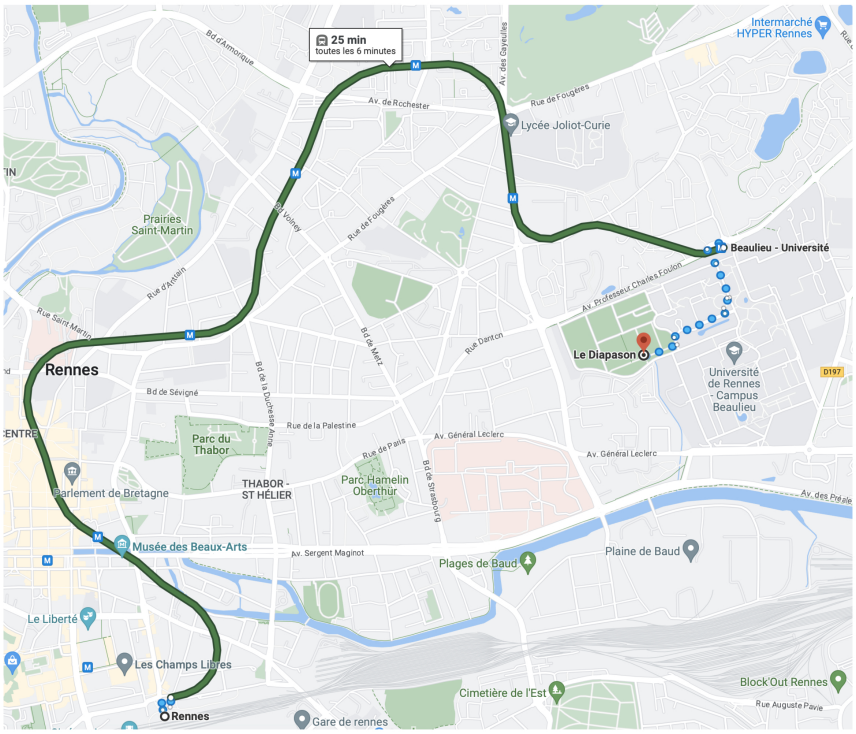

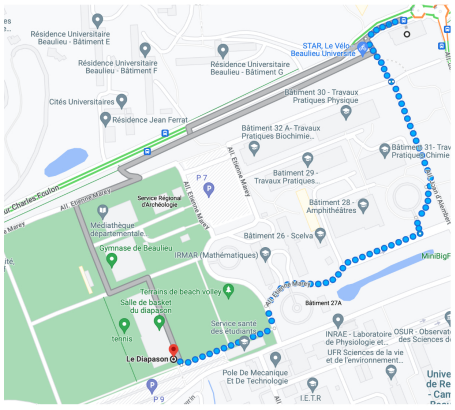

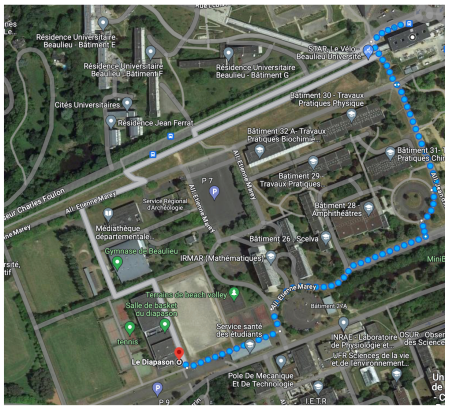

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