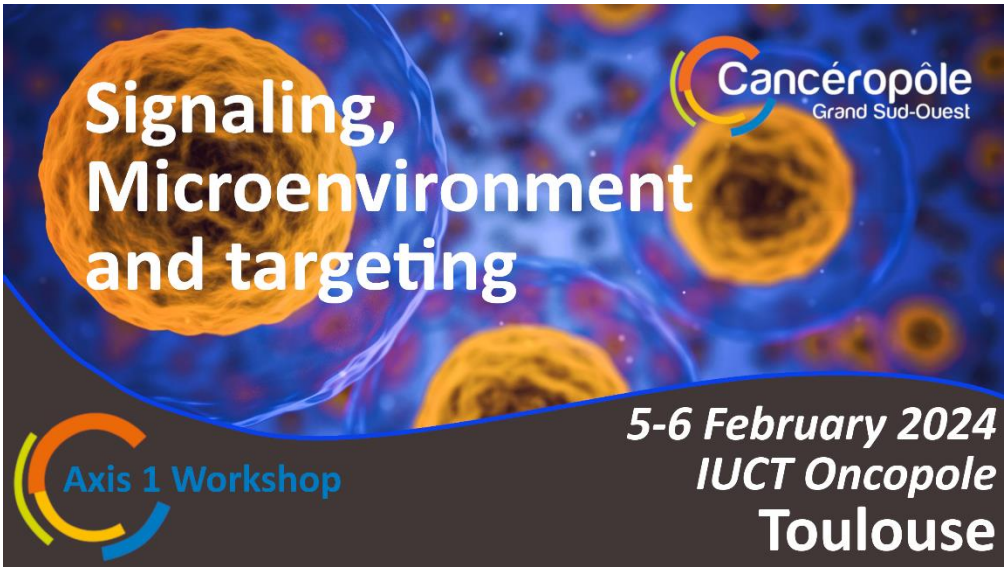
Cancéropôle Grand Sud-Ouest

Workshop
"Signaling, Microenvironment and Targeting"

Toulouse, February 5-6 2024



Axis 1

Program

February 5th, 2024

Monday February 5th, 2024	
12h45	**Welcome coffee & CRCT Visit *(upon registration)***
13h50	**Welcome address**
14h00	**Session 1 – Plasticity and heterogeneity** *Chairs: Violaine MOREAU & Aubin PENNA*
14h00	**Lecture: The role of ExtraCellular Matrix in melanoma cell plasticity and resistance to targeted therapy,** **Sophie TARTARE-DECKERT**, Centre Méditerranéen de Médecine Moléculaire, Nice
14h40	Mechanics & genetics of pancreatic cancer mechanical compressive stress favors selective mutational contexts and intracellular signaling during pancreatic cancer development **Mickael DI-LUOFFO**, CRCT, Toulouse
14h55	Single-cell quantitative phosphoproteomics analysis of cellular heterogeneity to predict melanoma response to MAPK inhibitors **Florian FAVIER**, IRCM, Montpellier
15h10	Multi-OMICS to unveil the role of MDM4 and lipid metabolism in the control of cellular plasticity in melanoma **Lisa MARY**, CRCT, Toulouse
15h25	Endothelins positively influences the proliferation vs migration balance of glioma stem cells and promotes proneural to astro-mesenchymal transition **Jean-Philippe HUGNOT**, IGF, Montpellier
15h45	**Coffee & posters' session**
16h50	**Session 2 – Signaling, Crosstalk & Resistance** *Chairs: Antonio MARAVER & Dennis GOMEZ*
16h50	Microbiota-gut-brain axis in glioblastoma development and therapeutic resistance **Océane MARTIN**, IBGC, Bordeaux
17h10	Uncovering phenotypic heterogeneity of drug tolerance in oncogene-addicted non-small cell lung cancer **Celia DELAHAYE**, CRCT, Toulouse
17h25	A metabolic crosstalk occurs between human bone marrow adipocytes and prostate cancer cells **Marine HERNANDEZ**, IPBS, Toulouse
17h40	Trogocytosis of cancer-associated fibroblasts promotes pancreatic cancer growth and immune suppression via phospholipid scramblase anoctamin 6 (ANO6) **Charline OGIER**, CRCT, Toulouse
17h55	Oncogenic signaling of PEAK pseudokinases in human cancer **Serge ROCHE**, CRBM, Montpellier
18h15	Tissue Resident Memory T cells dictate patients' prognosis and mediate immune checkpoint responses in colorectal cancer liver metastases **Syrine ABDELJAOUED**, UMR 1098 RIGHT, Besançon
18h40	**End of day 1**
20h00	**"Apéritif dînatoire" – The Botanist Pub *(upon registration)***

Cancéropôle Grand Sud-Ouest

February 6th, 2024

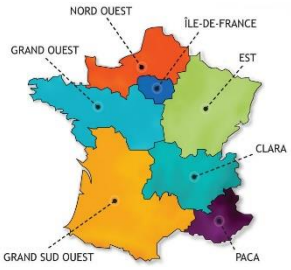
Tuesday February 6th, 2024	
9h00	**Session 3 – Tumor microenvironment: cellular interactions and immunity** *Chairs: Mary POUPOT & Nicolas LARMONIER*
09h00	Co-stimulatory Receptors in Action: Shaping the Immunological Synapse of Cytotoxic T Cells for Target Cell Elimination **Loïc DUPRE**, Infinity, Toulouse
09h20	Innate CD8 T-cells are part of tissue resident-memory (TRM) CD8 T-cells with an anti-tumoral signature in ovarian cancer **Alice BARBARIN**, IRMETIST, Poitiers
09h35	Role of MAP3K8/COT in T-cell mediated anti-tumor immune responses **Virginie MIEULET**, Infinity, Toulouse
09h55	Revisiting the role of CXCR2 and neutrophils in breast cancer **Gwendal LAZENNEC**, SYS2DIAG, Montpellier
10h15	Cancer-associated fibroblast spatial heterogeneity and EMILIN1 expression in the tumor microenvironment modulate TGF-β activity and CD8+ T-cell infiltration in breast cancer **Andreï TURTOI**, IRCM, Montpellier
10h40	**Coffee & posters' session**
11h40	**Session 4 – Pathophysiology and Treatments** *Chair: Guillaume BOSSIS*
11h40	Modelling cancer associated cachexia **Alexandre DJIANE**, IRCM, Montpellier
12h00	Identification of leukemic stem cells in a novel model of chronic myeloid leukemia **Catherine SAWAI**, BRIC, Bordeaux
12h20	SUMOylation Controls AML Cells Migration Through the Regulation of CD36 Gene Expression **Dana AKL**, IGMM, Montpellier
12h35	Unraveling the role of Polypyrimidine Tract Binding Protein 1 (PTBP1) in Acute Myeloid Leukemia: at the cross-road between metabolism, proliferation and survival **Margherita GHISI**, CRCT, Toulouse
13h00	**End of the workshop & Cocktail lunch**
14h00	**CRCT Visit *(upon registration)***

MEETING ORGANIZERS

Scientific organizers - Axis 1 "Signaling, Microenvironment and Targeting" Steering Committee

Barbara Bessette (CAPTuR, Limoges), **Guillaume Bossis (IGMM, Montpellier)**, Dennis Gomez (IPBS, Toulouse), Nicolas Larmonier (ImmunoConcEpT, Bordeaux), Antonio Maraver (IRCM, Montpellier), **Violaine Moreau (BRIC, Bordeaux)**, Aubin Penna (4CS, Poitiers), Mary Poupot (CRCT, Toulouse), Christophe Sirac (CRIBL, Limoges), François Vergez (CRCT, Toulouse).

The French Cancéropôles network and the Cancéropôle Grand Sud-Ouest



The 7 Canceropôles have been created in 2003 and are supported by the French Cancer Institute (INCa).They are part of the French cancer research landscape, enabling at regional and inter-regional levels a better coordination of skills and resources, while breaking down barriers between sectors and disciplines. They give rise to large-scale multidisciplinary research networks gathering research teams from research institutions, university hospitals, cancer centers, pharmaceutical and biotech companies, as well as dedicated stakeholders.

Cancéropôle Grand Sud-Ouest is a consortium bringing together more than 300 scientific and medical research teams in 2 regions of the Great South-West of France (Nouvelle Aquitaine and Occitanie). Cancéropôle Grand Sud-Ouest provides a federative ground and brings together all the institutions involved in cancer research to initiate dynamic networking and build federative research or technological programs, on a voluntary and collaborative basis. Participating fundamental and clinical research teams are attached to national research organisations (mainly INSERM and CNRS), 6 universities, 6 university hospitals (CHU) and 3 comprehensive cancer centres (CLCC). Private partners are also closely involved in the consortium (Biotechs, Big Pharmas). Cancéropôle Grand Sud-Ouest is constituted as a GIP (Groupement d'Intérêt Public), and its management is ensured by a president and a scientific director, appointed for 3 years. They combine the legal, administrative, and financial responsibilities and the direction of its scientific strategy. Cancéropôle Grand Sud-Ouest is mainly financed by the French National Cancer Institute.

Cancéropôle Grand Sud-Ouest has developed a dynamic network of skills by the animation of research axis, interdisciplinary disease-oriented consortia and the support of workgroups sharing technological approaches and competencies. Expertise covers all areas of cancer research, from basic research to translational research, also integrating the human and social sciences and technological approaches. Cancéropôle Grand Sud-Ouest's funding enables the emergence of innovative projects, the support of technological platforms, the organization of scientific seminars and specific trainings.

For the 2023-2027 period, the Cancéropôle Grand Sud-Ouest has decided to set up a scientific strategy focused on cross-cutting scientific themes, in full evolution and essential in a near future. The following 5 themes have been selected: Spatial biology, Chemistry and Cancer, Environmental stress, Alternative models to animal experimentation and, Methods, Management and Data analysis. These themes will allow to promote multidisciplinary approaches and collaborative projects, to improve the readability and promotion of local research groups and consortia in the country and sharing of existing expertise in the Greater Southwest, to strengthen them and to facilitate the emergence of new technologies. It should be noted that interfaces will also be developed between these themes.

Such an organization makes it possible to enhance team performance and increase synergies between researchers, clinicians and industry, across disciplines and territories.

Cancéropôle GSO

✉ 5 avenue Irène Joliot Curie, 31100 Toulouse

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www.canceropole-gso.org

LE PROGRAMME DE SOUTIEN A L'EMERGENCE DU CANCEROPOLE GSO

OUVERTURE LE 5 FEVRIER 2024 - SOUMISSION EN LIGNE

EMERGENCE DE PROJETS

OBJECTIFS	Valider les premières étapes d'un projet ou une étude de faisabilité indispensables pour une soumission à un AAP national
CRITERES	Approche nouvelle et originale, nouvelle voie d'exploration ou arrivée d'une équipe dans un nouveau champ disciplinaire
FINANCEMENT	25 k€ maximum par projet

EMERGENCE DE MODELES ET OUTILS

OBJECTIF	Soutenir la mise en place de modèles et outils innovants avec une visée technique, allant des modèles biologiques à la modélisation et au traitement des données en lien avec le cancer, afin de favoriser la mise à disposition de nouveaux modèles pour la communauté du GSO et servir de tremplin pour l'obtention de financements plus importants
CRITERES	Approche nouvelle et originale, impact du développement d'un tel modèle/outil en cancérologie
FINANCEMENT	25 k€ maximum par projet

LES PROGRAMMES DE SOUTIEN, SOUMISSION EN LIGNE AU FIL DE L'EAU

MOBILITE TECHNOLOGIQUE

OBJECTIF	Acquérir une technologie originale, qu'elle soit ou non déjà présente dans le GSO.
PUBLIC ELIGIBLE	Statutaires, doctorants en 1ère et 2ème année et non-statutaires (les non statutaires doivent s'engager à rester dans leur équipe de recherche au minimum pendant 12 mois après la fin de la mobilité).
SEJOUR	3 mois maximum — FINANCEMENT 4 k€ maximum

ORGANISATION DE SEMINAIRES

CRITERES	Séminaires organisés sur le territoire du GSO et ouverts à l'ensemble de la communauté scientifique du GSO.
FINANCEMENT	2 k€ maximum sous forme de subvention, de prise en charge d'un conférencier ou d'inscriptions d'étudiants et de jeunes chercheurs.

SOUMISSION AU MINIMUM 4 MOIS AVANT LA DATE DE L'EVENEMENT

COLLABORATION TRANSFRONTALIERE

OBJECTIF	Organiser la réunion d'équipes de recherche afin de construire un projet de recherche et servir de tremplin pour l'obtention de financements.
PAYS ELIGIBLES	Pays du Sud-Ouest européen : Espagne et Portugal.
FINANCEMENT	4 k€ maximum pour un déplacement ou un cycle de déplacements sur une période d'un an impliquant plusieurs chercheurs du GSO.

The role of ExtraCellular Matrix in melanoma cell plasticity and resistance to targeted therapy

Sophie TARTARE-DECKERT

Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U1065, Université Côte d'Azur, team "Microenvironment, Signaling and Cancer", Nice, France

Our laboratory is interested in understanding microenvironmental influences and signaling networks that drive tumor growth and dissemination. We have been particularly involved in studying the role of tumor microenvironment in metastatic niche formation and response to therapies in melanoma, the most aggressive and lethal form of skin cancers. The matricellular protein SPARC is an important driver of theses processes, particularly by inducing mesenchymal phenotypic transition, tumor cell extravasation and p53-dependent survival. Our current work focuses in deciphering how the extracellular matrix (ECM), a key component of the microenvironment shapes the response of melanoma cells to therapy targeting the BRAFV600 oncogenic pathway or checkpoint blockade immunotherapy, and tumor cell plasticity. I will first present an overview of our recent findings showing that MAPK-targeted therapy induces a fibrotic-like response associated melanoma cell mesenchymal dedifferentiation, ECM remodeling, and tumor stiffening. I will also present data showing how we can therapeutically manipulate tumor-associated fibrosis and ECM-mediated signaling to overcome therapy resistance and delay tumor relapse. Finally, I will present new data showing that melanoma cell plasticity dictates the response to extracellular mechanical signals with the functional involvement of DDR1/2 collagen receptors, and that mechanical addiction of the dedifferentiated melanoma cell state represents a new vulnerability for this aggressive and therapy resistant phenotype.

Selected publications

Tichet et al. Nat Commun 2015 ; Rathore et al. Oncogene 2019
Girard et al. Cancer Res 2020 ; Diazzi et al. EMBO Mol Med 2022
Berestjuk et al. EMBO Mol Med 2022 ; Popovic & Tartare-Deckert. Front Oncol 2022
Rovera et al. Cancer Res 2022 ; Diazzi et al. Oncogenesis 2023

Mechanics & Genetics of pancreatic cancer Mechanical compressive stress favors selective mutational contexts and intracellular signaling during pancreatic cancer development

Mickael DI-LUOFFO[1], Silvia ARCUCCI[1], Tristan MARTY[1,2], Nicole THERVILLE[1], Sanzhar AITBAY[1], Pauline LEFEBVRE[1], Benoit THIBAULT[1], Pascal SWIDER[2], Pauline ASSEMAT[2], Morgan DELARUE[1,3], Julie GUILLERMET-GUIBERT[1,3]

[1] CRCT, U1037, INSERM-CNRS
[2] IMFT, UMR 5502, CNRS-INP Toulouse-UT3 Paul Sabatier
[3] LAAS, UPR8001, CNRS

Context: Mechanical compressive stress arises during pancreatic cancer progression (PDAC)1. In vitro, compression forces decrease PDAC cell proliferation, increase invasiveness, and induce resistance to chemotherapies. In vivo, the importance of compression is unknown. In PDAC, increased compressive stress happens simultaneously with the second wave of genetic alterations (p53 mutations/truncations) after KRAS oncogenic mutations; it is also linked with overexpression/activation of the PI3-Kinases (PI3K) pathway2. We think that compression favors selective genetic backgrounds that modify the signaling environment in cells and thus cell fate.
Experimental design: We generated compressive stresses to spheroids derived from PDAC cells with KRASG12D mutation, in which p53R172H mutation or p53R172H;R210* truncation are induced sequentially. Further, we applied a compressive stress to KRASG12D±p53R172H/p53R172H;R210* mutated mouse allografts using a compressive device. We also used the punch method in order to evaluate the relaxation of tumors depending on their genetic background.
Results: Compression decreased the spheroid growth (<30%)3. However, p53R172H mutated PDAC cells developed a resistance to compression and continued to proliferate. This mutation associated with a truncation of p53 accentuated this resistance, even bringing a proliferative advantage. A transcriptomic analysis of KRASG12D, p53 mutated and p53 truncated spheroids under compression was performed. This analysis showed a modification in adhesion properties via plasma membrane and RTK signaling activity, mechanisms regulated by PI3K pathway. In parallel, we observed, in vivo, that the growth of KRASG12D mutated tumors decreased by 40% under compression, whereas the size of tumors with p53R172H;R210* truncated form was similar with or without compression. Finally, KRASG12D tumors relaxed more easily compared to the p53R172H and p53R172H;R210* tumors; this was due to a greater cellular and matrix homogeneity in these tumors compared to p53R172H and p53R172H;R210* tumors.

Conclusion: Growth under pressure can influence the progression of PDAC promoting selective genetic background and activation of oncogenic signaling pathways. These observations open the way to integrate the mechanical context in the management of patients with PDAC.

1 Northcott, J. M., Dean, I. S., Mouw, J. K. & Weaver, V. M. Feeling Stress: The Mechanics of Cancer Progression and Aggression. Front Cell Dev Biol 6, 17, doi:10.3389/fcell.2018.00017 (2018).
2 Di-Luoffo, M., Ben-Meriem, Z., Lefebvre, P., Delarue, M. & Guillermet-Guibert, J. PI3K functions as a hub in mechanotransduction. Trends Biochem Sci, doi:10.1016/j.tibs.2021.05.005 (2021).
3 Rizzuti, I. F. et al. Mechanical Control of Cell Proliferation Increases Resistance to Chemotherapeutic Agents. Phys Rev Lett 125, 128103, doi:10.1103/PhysRevLett.125.128103 (2020).

Single-cell quantitative phosphoproteomics analysis of cellular heterogeneity to predict melanoma response to MAPK inhibitors

Florian FAVIER, Eulalie CORRE, Candi STUMPF-LÉONARD, Yaël GLASSON, Henri-Alexandre MICHAUD, Alain MANGÉ, Romain LARIVE

IRCM, Université de Montpellier, ICM, INSERM, Montpellier, France.

Advanced or metastatic melanoma, an aggressive form of skin cancer, represents a major challenge in the field of oncology. In 50% of cases, BRAF kinase mutations play a significant role in the initiation and progression of this disease, by impacting the MAPK mitogenic signaling network. The use of BRAF and MEK kinase inhibitors has revolutionized the treatment of BRAF-mutated metastatic melanoma, bringing impressive clinical responses. However, a quarter of patients do not respond to these treatments. What's more, in most responders, due to the great plasticity and heterogeneity of melanoma, tumors eventually develop resistance via a variety of mechanisms, leading to recurrence. These resistance mechanisms almost invariably lead to the reactivation of MAPK and/or PI3K/AKT signalling networks, making these networks central players in the resistance of melanoma to targeted kinase inhibitor therapies. Our hypothesis is that primary and acquired resistance to therapies may be predetermined by biomarkers linked to the initial state of these intracellular molecular networks. In addition, various studies have shown that certain subpopulations of melanoma cells are less sensitive to MAPK inhibitors, and are therefore the reservoir of the persistent cells that constitute pre-recurrent residual disease.Our aim is to analyze the heterogeneity and plasticity of the MAPK signaling network to predict the response of metastatic melanoma to current clinical inhibitors. To this end, we have developed a new technique for quantitative single-cell phosphoproteomic analysis using mass cytometry (CyTOF). We optimized the experimental protocol for adherent cells and to preserve the phosphorylation state of the proteins. We built a panel of antibodies to quantitatively analyze 28 phosphoproteins of signaling networks, 6 markers of melanoma subpopulations and 6 markers of proliferation, apoptosis and cell cycle. Finally, we barcoded the cells for simultaneous analysis of the 451Lu cell line, sensitive or resistant version, disrupted by 7 inhibitors over short or medium timescales. At the scale of overall cell populations, comparison of sensitive and resistant cells shows a change in cell identity accompanied by drastic remodeling of MAPK and PI3K/AKT networks. At the single-cell level, we detected several subpopulations among sensitive cells with MAPK and PI3K/AKT networks distinct from the majority population, demonstrating that these networks and how they are disrupted by inhibitors are heterogeneous at the cellular level. By following inhibitor-treated cells for several days, we noticed an early adaptation of some cells to inhibitors, suggesting their ability to survive as persistent cells thanks to their molecular plasticity. These results are very encouraging and demonstrate the benefits of studying phosphoproteomics at the single-cell level. We now need to associate this molecular heterogeneity with the subpopulations of melanoma cells responsible for residual disease, and determine how they contribute to the acquisition of resistance. [This work has been supported by the Fondation ARC pour la recherche sur le cancer (Projets ARC 2022 PJA2 N°ARCPJA2022060005107), the Ligue nationale contre le cancer (Comité du Gard JPB/GA/MV/39-2019) and the INCa-Cancéropôle GSO (Programme Emergence N°2018-E1). FF is supported by the Ligue nationale contre le cancer (Financement THÈSE 1ère année 2023 N°IP/SC - 18179)].

Multi-OMICS to unveil the role of MDM4 and lipid metabolism in the control of cellular plasticity in melanoma

Lisa MARY[1], Elodie MUCHER[1], Virginie GARCIA[1], Gemma FABRIAS[2], Nathalie ANDRIEU-ABADIE[1]

[1] Centre de Recherche en Cancérologie de Toulouse
[2] Institute of Advanced Chemistry of Catalonia

Metastatic melanoma is the deadliest skin cancer. Understanding the molecular mechanisms behind the resistance to treatments (targeted therapy, immunotherapy) is a major challenge. Phenotypic plasticity, which is associated with an increased capacity to adapt to the surrounding stress and abilities for invasion and survival, is now accepted to be the main source of resistance in melanoma. Multiple genes could be involved in the control of tumor plasticity, including TP53 encoding the p53 protein. TP53 is weakly mutated in patients with melanoma, however the protein is inactivated in approximately 90% of them. This inactivation is the consequence of an amplification of negative regulators including MDM4 (Mouse Double Minute 4). Here, we show that MDM4 silencing restores p53-mediated cell-cycle slowing and senescence. Reactivation of p53 also affects the plasticity of melanoma cells as well as their migratory potential through a mechanism depending, in part, on ceramide metabolism. Overall, these results suggest that, in combination with current therapies, MDM4 could become a major therapeutic target in the treatment of metastatic melanoma.

Endothelins positively influences the proliferation vs migration balance of glioma stem cells and promotes proneural to astro-mesenchymal transition

Jean-Philippe HUGNOT[1], D. PINEAU[1], L. GARCIA[1], K. AGUILAR-CAZAREZ[1], C. RIPOLL[1], S. HIDEG[1], D. BOQUET[1], V. ASEI-CESCHINO[2], L. BAUCHET[1,3], V. RIGAU[1,3], Jp. PIN[1], P. RONDARD[1], L. PREZEAU[1], H. DUFFAU[1,3]

[1] Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, Montpellier, France
[2] Institut Génétique Humaine, Université de Montpellier, CNRS, INSERM, Montpellier, France
[3] Montpellier Hospital, CHU, Neurosurgery department, Montpellier, France

Endothelins, a family of cytokines prominently expressed by endothelial cells, exert their effects through binding to two G-protein-coupled receptors, EDNRA and EDNRB. Despite their acknowledged involvement in various physiological processes, the precise role of endothelin signaling in gliomas and glioblastoma stem cells remains unclear and controversial. In this study, we systematically investigated this signaling pathway utilizing a glioma cell line biobank containing lines at different malignancy stages, along with specimens obtained from glioma patients.

Our findings reveal a distinct expression pattern, with EDNRB prevailing over EDNRA, and a reduction in EDNRB expression as gliomas progress in malignancy. Notably, glioma stem cells exhibit a capacity to adopt mesenchymal-astrocyte-like and proneural oligodendrocyte-like phenotypes upon differentiation, and EDNRB is predominantly expressed in the astrocyte-like state, both in vitro and in vivo. Treatment of glioma stem cell cultures with endothelins and EDNRB agonists resulted in a noteworthy reduction in proliferation accompanied by an increase in cell mobility. Furthermore, comprehensive signaling analyses uncovered the activation of multiple pathways, including STAT3, Hippo/YAP, Ca2+, and K+ signaling, following endothelin stimulation in glioma stem cells. Employing a multiomics approach, we observed that endothelins drive glioma stem cells toward a mesenchymal-astrocyte-like phenotype at the expense of the proneural oligodendrocyte-like state.

In summary, our study sheds light on the pivotal role of endothelin signaling in glioma stem cells, underscoring its significance as a key regulator of cell proliferation and the transition towards mesenchymal-astrocyte-like states.

Microbiota-gut-brain axis in glioblastoma development and therapeutic resistance

Gauthier DELROT[1], Sarah LAVIELLE[1], Marie-Alix DERIEPPE[2], Julie MARTINEAU[2], Marcia CAMPISTRON[2], Manon LEMAITRE[1], Jean DESCARPENTRIE[3], Alexandra GRANGER-FARBOS[4], Benoit PINSON[4], Domitille CHALOPIN-FILLOT[1], Ioannis PATERAS[5], Macha NIKOLSKI[1], Thomas DAUBON[1], **Océane MARTIN CB[1]**

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[2] Animal Facility, University Bordeaux, Pessac, France;
[3] Department of Molecular Biology and Umeå Centre for Microbial Research (UCMR), Umeå University, Umeå, Sweden;
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Glioblastoma (GB) is the most common subtype of glioma in adults. Despite treatment through tumor resection associated with chemo and radiotherapies, this cancer still has a very poor prognosis. Factors contributing to etiology, pathogenesis, or treatment resistance are not well known. The importance of the microbiome-gut-brain axis (MGBA) has been recently shown in several neurodegenerative diseases. However, the role of the MGBA in GB has not been yet extensively studied.
In this study, we have examined the relationship between gut inflammation, microbiome modulation, and GB development and therapeutic resistance. Mice received dextran-sulfate sodium (DSS), a gut pro-inflammatory agent, and were orthotopically injected with mGB2 GB cells. Some mice were then resected and treated by radio and chemotherapy using Temozolomide (TMZ).

Our results showed that DSS-treated mice had a higher GB growth compared to non-treated mice. Moreover, the recurrence after treatment was higher in mice bearing gut inflammation. Interestingly, we also observed on DSS-treated mice, that the GB-bearing mice had lower intestinal inflammation compared to control. GB growth was also associated with microbiota modifications which were restored by the treatment. To understand the relationship between both gut and brain, we are currently analyzing the bloodstream metabolic content as well as the immune landscape in both organs. Finally, a bioinformatic approach will allow us to integrate our different data sets, such as systemic metabolic content, microbiome modulation, brain transcriptome, and tumor growth.

This study will allow us to gain insight into the relationship between the gut microbiome and pathophysiology and the brain in the context of GB development and treatments. Long term perspective would be to target gut microbiome in order to slow down GB progression and/or to improve treatment efficacy.

Uncovering phenotypic heterogeneity of drug tolerance in oncogene-addicted non-small cell lung cancer

Célia DELAHAYE[1], Sarah FIGAROL[1], Rémi GENCE[1], Aurélia DOUSSINE[1], Juan-Pablo CERRAYO[1], Estelle CLERMONT[1,2], Anne CASANOVA[1,2], Gilles FAVRE[1,2,3], Julien MAZIERES[1,3,4], Anne PRADINES[1,2], Olivier CALVAYRAC[1]

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[4] CHU de Toulouse

Targeted therapies are effective treatments for advanced lung cancer patients bearing oncogene drivers alterations, but are not curative due to the inevitable apparition of resistance. The resistant proliferative cells may arise from a small population of drug tolerant cells (DTC) through non-genetic reprogramming. Deciphering the vulnerabilities of these cells is therefore essential to propose new therapeutic strategies to patients, but the molecular mechanisms underlying drug tolerance is still poorly understood.

We performed an extensive step-by-step characterisation of the molecular and phenotypic processes involved in the adaptive response to targeted therapies in vitro and in Patient-Derived Xenograft (PDX) models, at the single cell level. We found that the DTC population is a highly dynamic and heterogeneous state, with both stably G1-arrested cells and early cycling escapers, that exhibit contractility features via Rho/ROCK-dependent actin cytoskeleton remodelling. We also shed light on the phenotypic switch that DTC undergo, as they transition to a pseudo-healthy lung phenotype under treatment, while acquiring mesenchymal characteristics as they develop resistance mechanisms. We identified a farnesyltransferase inhibitor compound that efficiently prevents relapse to targeted therapies in various cell lines, and strongly delays the acquisition of resistance in several PDX models. We are now investigating whether our characterisation of drug tolerance is relevant in patients, by following a cohort of 40 EGFR-mutated lung cancer patients treated with targeted therapy. We are using liquid biopsies to monitor circulating tumour DNA and characterise circulating tumour cells during treatment.

A metabolic crosstalk occurs between human bone marrow adipocytes and prostate cancer cells

Marine HERNANDEZ[1], Nicolas REINA[2], Catherine MULLER[1], Camille ATTANE[1]

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In localized prostate cancer (PCa), we have demonstrated that periprostatic adipocytes increase tumor progression by providing cancer cells with fatty acids (FAs) released after the activation of lipolysis, involving the hydrolysis of triglycerides (TG) [1]. In advanced PCa, the majority of metastases are found within the bone, where tumor cells can interact with bone marrow adipocytes (BMAds). However, whether a metabolic crosstalk between primary BMAds and PCa exists and favors tumor progression remains to be determined. Thanks to a collaboration with orthopedic surgeons, we obtain human bone marrow adipose tissue (BMAT) during hip replacement surgery. There are two types of BM-Ads: those contained in the red BMAT (rBMAds) and those contained in the yellow BMAT (yBMAds), which have been characterized by my team [2]. Since PCa metastatic sites are frequently found in proximity to rBMAds, we established a 3D culture of these adipocytes in a fibrin matrix to preserve their viability for up to 5 days and cultured them with PCa cells.

Under coculture conditions, PCa cells exhibited an increase in neutral lipid content, primarily composed of TG. Using rBMAds loaded with fluorescent FAs, we directly demonstrated that FAs released by rBMAds are taken up by cancer cells and re-esterified into TG. These data provide the first evidence of a metabolic crosstalk between primary human rBMAds and PCa cells. Through lipidomic approaches, we determined that rBMAds release FAs mainly palmitate, oleate, and linoleate. However, like yBMAds [2], we found that rBMAds are devoid of lipolysis due to a profound decrease in the expression of the last two enzymes of the lipolytic pathway. These data suggest that an original mechanism, independent of classical lipolysis, may be involved in the release of FAs by rBMAds. Interestingly, the first lipolytic enzyme, ATGL (Adipose Triglyceride Lipase), and its cofactor are expressed in rBMAds and could participate in the release of FAs through an unusual incomplete lipolytic process which is currently under investigation. Once inside tumor cells, we found that FAs are stored as TG but are also oxidized in mitochondria. However, this increased fatty acid oxidation is not associated with increased ATP production. Thus, FAs taken up by PCa cells are not primarily used for energy production but could be involved in other processes, such as transcriptomic remodeling. RNASeq and gene ontology analyses of PCa cells cocultivated with or without rBMAds reveal clear differences in migration pathways. This process is a key step in the propagation of cancer cells from one bone metastatic site to other bone metastatic site, making the disease highly aggressive. Functional experiments confirmed that rBMAds specifically increase the migratory capacity of different PCa cell lines without any increase in proliferation. Whether this pro-migratory effect of rBMAds is due to the transfer of FAs is under investigation.

In conclusion, the metabolic crosstalk between rBMAds and PCa cells could contribute to the propagation of bone metastasis. Deciphering this crosstalk, including other metabolites, could lead to pharmacological targets for the treatment of bone metastases, for which therapeutic options remain very limited.

[1] Laurent V, Toulet A, Attané C, et al. Periprostatic Adipose Tissue Favors Prostate Cancer Cell Invasion in an Obesity-Dependent Manner: Role of Oxidative Stress. Mol Cancer Res. 2019;17(3):821-835. doi:10.1158/1541-7786.MCR-18-0748
[2] Attané C, Estève D, Chaoui K, et al. Human Bone Marrow Is Comprised of Adipocytes with Specific Lipid Metabolism. Cell Rep. 2020;30(4):949-958.e6. doi:10.1016/j.celrep.2019.12.089

Trogocytosis of cancer-associated fibroblasts promotes pancreatic cancer growth and immune suppression via phospholipid scramblase anoctamin 6 (ANO6)

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The fibroblastic stroma comprises most of pancreatic adenocarcinoma mass and is remarkably devoid of functional blood vessels leaving an unresolved question of how pancreatic cancer cells obtain their essential metabolites and especially water-insoluble lipids. Contrary to the previously held assumption that cancer cells uptake lipids directly from the interstitial fluid, we have found a critical role for cancer-associated fibroblasts (CAFs) to obtain and transfer blood-borne lipid particles to cancer cells via trogocytosis, a process of "nibbling" of plasma membranes between two cells engaged in synapse-like membrane contacts. Whereas trogocytosis has been described in normal development, the biochemical and signaling regulators of trogocytosis between CAFs and PDAC cells have not been defined.

We determined that CAF membrane trogocytosis is triggered by externalized phosphatidylserine (PtdSer), and blockade of PtdSer in vitro transiently deters trogocytic uptake of CAF membranes. We have also discovered a phospholipid scramblase anoctamin 6 (ANO6) expressed in CAFs as the essential trogocytosis regulator to promote cancer cell survival. Mechanistically, CAF-cancer cell membrane contacts induce cytosolic calcium influx via Orai channels, which activates ANO6 and results in phosphatidylserine exposure on CAFs. As a promising therapy target, ANO6 protein is highly expressed in PDAC tumor mass in cancer cells, endothelial cells and CAFs and is a negative prognostic biomarker for survival. Depletion of ANO6 in co-implanted CAFs dramatically reduced the growth of orthotopic pancreatic tumor grafts. Furthermore, pharmacologic inhibitors of ANO6 with clinically available antibiotics niclosamide or clofazimine potently blocked cholesterol uptake in vivo by PDAC cells.

Our findings indicate a novel trogocytosis function for CAFs in highly desmoplastic carcinomas as the main mechanism of lipids delivery to cancer cells. CAFs do so by expressing PtdSer as "eat me" signals. This process is regulated by Ca2+-dependent phospholipid scramblase ANO6. Re-purposing of clinically available ANO6 inhibitors could make a tangible impact on treatment of PDAC patients in the near term.

Oncogenic signaling of PEAK pseudo-kinases in human cancer

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Inactive kinases have gained attention in recent years because they play a crucial role in cancer, similar to active kinases. Understanding how these pseudo-kinases promote tumor formation despite their catalytic inactivity is a significant challenge. This understanding may lead to innovative anti-cancer therapies. We and others have recently linked pseudopodium-enriched atypical kinase (PEAK) 1/2, also known as Pragmin/SGK223/NACK/PEAK1 and PEAK2, with the progression of epithelial cancers such as breast and colon cancer. Several reports, including our own, have described a model that explains their assembly and regulation of oncogenic signalling pathways through structural analysis. More recently, we have identified C19orf35/PEAK3, a missing gene of the human kinome, as a novel member of this family and an important promoter of acute myeloid leukaemia. We will present a unified model on how PEAK pseudo-kinases induce oncogenic signaling, leading to cancer cell proliferation and migration. Additionally, we will discuss potential therapeutic strategies to block their tumor activity based on these studies.

Tissue Resident Memory T cells dictate patients' prognosis and mediate immune checkpoint responses in colorectal cancer liver metastases

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Tissue resident memory (TRM) T cells have emerged as key players in cancer immunosurveillance, and their presence has been linked to a favorable clinical outcome in solid cancer patients. Liver metastases exhibit a highly immunosuppressive tumor microenvironment, however the role and clinical impact of TRM cells infiltration in colorectal cancer remain elusive. An exhaustive profiling was conducted on tumor infiltrating lymphocytes isolated from patients' colorectal cancer liver metastases (CRC-LM) and compared to peripheral blood samples of CRC-LM patients. Cytokine production was also evaluated in in vitro activated TRM and non-TRM cells. Prognostic value of TRM cells was also assessed in a well-defined cohort of CRC-LM. Here we identified two subsets of TRM cells expressing CD103 and/or CD69 showing significantly higher expression of tissue residency and activation biomarkers. CD103+CD69+ TRM cells subset showed almost exclusive expression of tumor reactivity biomarkers PD-1 and CD39. Supporting this observation, CD103+CD69+ TRM cells showed a more oligoclonal TCR repertoire. Both TRM subsets presented higher cytotoxic and functional capacity compared to non-TRM cells. Our study shows that only the presence of CD103+CD69+ TRM cells is associated with longer recurrence free survival of colorectal cancer patients with liver metastases and associated with response to immune checkpoint inhibitors in MSIhigh patients. Taken together, our work demonstrates the existence of a phenotypic heterogeneity of TRM cells in colorectal cancer liver metastases. In this study, we identified a population of CD103+CD69+ TRM cells exhibiting the characteristics of tumor reactivity and correlated with better patients' prognosis, with potential implication in optimal therapeutic strategies determination.

Co-stimulatory Receptors in Action: Shaping the Immunological Synapse of Cytotoxic T Cells for Target Cell Elimination

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Immunotherapies based on the reinvigoration of T cell responses via immune checkpoint blockers or on the administration of engineered T cells containing chimeric antigen receptors (CAR-T cells) are revolutionizing the treatment of cancer. Yet the efficacy of T cell-based therapies remains suboptimal for multiple cancers and not all treated patients benefit from long-lasting control of their tumor. The ability of T cells, and particularly CD8+ cytotoxic T lymphocytes, to eliminate tumor target cells relies not only on the recognition of tumor-associated antigens through the TCR, but also on the balance of stimulatory and inhibitory signals transmitted via co-stimulatory and co-inhibitory receptors. The knowledge of the mechanisms by which co-inhibitory receptors such as PD1 and CTLA4 hamper T cell activation and function has been successfully exploited for the design of immune checkpoint therapies. On the other hand, the possibility to stimulate anti-tumor T cell activity via co-stimulatory receptors has lagged behind. This is in part due to the current lack of knowledge on how these receptors modulate cytotoxic T cell function. Indeed, although co-stimulatory receptors are known to amplify TCR signaling, their impact on the multiple steps that govern the cytotoxic activity has not been systematically assessed. Given the diversity of co-stimulatory receptors and their distinct signaling modules, we have hypothesized in this work that these receptors might differentially affect the multiple steps occurring at the immunological synapse formed between T lymphocytes and target cells.

In order to systematically compare the impact of co-stimulatory receptors engagement on various facets of immunological synapse architecture and function, we have applied a high-content/high-throughput confocal microscopy analysis to human CD8+ effector T cells co-expressing homogenous levels of three receptors of the Ig superfamily (ICOS, CD2 and CD226) and three receptors of the TNF-R superfamily (CD27, OX40 and GITR). Co-stimulation via CD2, ICOS or CD226 resulted in a substantial increase of cytotoxicity towards target cells, especially in conditions of low TCR triggering. While the engagement of OX40 and GITR failed to modulate cytotoxicity, CD27 engagement reduced cytotoxicity. Further analysis of the synaptic steps controlling cytotoxicity revealed that each co-stimulatory receptor modulated T cell activation in its own way. Remarkably, distinct immunological synapse architectures were imprinted by each co-stimulatory receptor. In particular, the engagement of CD226, ICOS or CD2 enhanced IS spreading through the formation of unique actin-rich protrusions. Notably, CD226 stimulation promoted the formation of a high-affinity LFA-1 adhesive belt. Consistent with this patterning, CD226 increased T cell adherence to target cells to facilitate killing. Furthermore, co-stimulatory receptor engagement affected, to varying degrees, the polarization of the microtubule-organizing center and the distribution of lytic granules at the synaptic area.

Our systematic analysis not only provides a classification of co-stimulatory receptors in terms of their potential to modulate cytotoxic activity, but also reveals their precise impact on each of the steps governing this essential function. Currently, our focus is to apply these findings to the rational design of improved CAR-T therapies.

Innate CD8 T-cells are part of tissue resident-memory (TRM) CD8 T-cells with an anti-tumoral signature in ovarian cancer

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Ovarian cancer, frequently diagnosed late and at an advanced stage, is the fifth most deadly cancer in women, responsible for 43% of cancer deaths. The first strategies using immunotherapies (anti-PD1 therapy…) were disappointing and current strategies tend to combine immunotherapies with different inhibitors (PPAR, VEGF, MEK, etc…) to improve patient prognosis. Understanding the immunological context of ovarian cancer would likely make it possible to more satisfactorily select the groups of patients likely to benefit from these new treatments.

Our laboratory has been focusing on innate T lymphocytes or cells (ITC), which include iNKT cells, MAIT cells, γδ-T cells, and innate CD8 T-cells (CD8 ITC). ITCs are part of natural immunity, between well-known innate and adaptative immunity, and possess characteristics of both. Indeed, in addition to their response capacity after their TCR engagement, ITCs have an IFN-γ secretion function in response to pro-inflammatory cytokines IL-12, IL-18, or IL-33. ITCs have been recognized by us and others for their anti-tumor potential.

In patients suffering from ovarian cancer, we previously reported the presence of CD8 ITC (defined by the Eomes+ KIRs/NKG2A+ markers) among the T-cells of the tumor, peritoneal carcinomatosis, or neoplastic ascites. In the same patients, we documented local production (in the tumor, the carcinomatosis and/or the inflammatory peritoneum) of IL-33 and IL-12, but not IL-18, the latter being elevated in plasma. Alongside our results, the Jose R. Conejo-Garcia team recently demonstrated that the immunogenicity of ovarian cancer is carried by a small percentage of CD8 T-cells (13%) having a memory resident phenotype (TRM) of progenitor type (or stemness), identified by low expression of the transcription factor TCF1. These cells share characteristics with CD8 ITCs, one of them being expression of the Eomes transcription factor.

The main objective of our project is to determine whether CD8 ITCs locally carry a TRM signature directed against the tumor, and evaluate their activation/exhaustion and stemness status, by comparing them to other ITC populations, adaptive CD8 T-cells and NK cells. We analyzed immune cells in tumor samples, carcinosis samples, cells from ascites, and blood from ovarian cancer patients by multiparametric spectral flow cytometry.

Our results showed that CD8 ITCs account for 15% of total intra-tumoral CD8 T-cells and that the NKG2A+ subset of CD8 ITCs is enriched in tumor samples. We also showed that CD8 ITCs are part of TRM CD8 T-cells with properties compatible with their participation in the anti-tumoral response.

This work provides information regarding the anti-tumor role of ITCs in ovarian cancer. Precisely, the detailed analysis of TRM populations will ultimately guide the choice of therapeutic strategy or will lead to the definition of a new signature for predicting therapeutic success if it turns out that the CD8 ITCs constitute a potential target for implementation of immunotherapies.

Role of MAP3K8/COT in T-cell mediated anti-tumor immune responses

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Tumorigenic activation of Mitogen-Activated Protein Kinases (MAPKs) is central for translational reprogramming of cancer cells, as it allows rapid cell adaptation to highly dynamic environment by promoting selective mRNA translation. Amongst these MAPKs, we and others found that gain of function alterations of the MEK kinase MAP3K8 (also known as TLP-2/COT) constitute an alternative to BRAF mutations in cancer (Gruosso et al., 2015; Newman et al., 2019). In high-grade serous ovarian cancers (HGSOC) which are rarely mutated for BRAF, we found that accumulation of MAP3K8 protein correlates with MEK/ERK activation and poor patient outcome (Gruosso et al., 2015). Interestingly, constitutive activation of MAP3K8/MEK promotes tumour growth and confers a new translational landscape by regulating the assembly and activity of the translation initiation complex eIF4F. In addition, first analyses show that MAP3K8 expression by tumour-infiltrating T lymphocytes (TILs) suppresses anti-tumoral responses by decreasing tumour-site production of effector molecules. Indeed, proliferation, activation and tumour-site production of effector molecules by TILs increases locally in the tumours upon Map3k8 deletion suggesting that MAP3K8 might restrain T cell functions after cancer cell priming by controlling translation of selected mRNA targets. By using knock out (KO) mouse models, we are now uncovering the intrinsic function of MAP3K8 in TILs and assess changes in the MAPK pathway and mRNA translational programs involved in cancer progression. Deciphering the crosstalk of cancer cells and TILs at the level of mRNA translation will be the basis of future combination therapies enhancing immune checkpoint inhibitor efficacy by targeting MAP3K8.

Gruosso, T., Garnier, C., Abelanet, S., Kieffer, Y., Lemesre, V., Bellanger, D., Bieche, I., Marangoni, E., Sastre-Garau, X., Mieulet, V., and Mechta-Grigoriou, F. (2015). MAP3K8/TPL-2/COT is a potential predictive marker for MEK inhibitor treatment in high-grade serous ovarian carcinomas. Nature communications 6, 1-15. 10.1038/ncomms9583.
Newman, S., Fan, L., Pribnow, A., Silkov, A., Rice, S.V., Lee, S., Shao, Y., Shaner, B., Mulder, H., Nakitandwe, J., et al. (2019). Clinical genome sequencing uncovers potentially targetable truncations and fusions of MAP3K8 in spitzoid and other melanomas. Nat Med 25, 597-602. 10.1038/s41591-019-0373-y.

Revisiting the role of CXCR2 and neutrophils in breast cancer

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Recent work has shown the possible involvement of tumor associated neutrophils (TANs) in tumor progression. Moreover, the high expression of the chemokine receptor CXCR2 in neutrophils appear crucial for their function. By crossing the PyMT mouse model of breast cancer with CXCR2 KO animals, we show that that KO display an enhanced tumor growth, due to a higher recruitment of neutrophils and a decreased number of macrophages in the tumor. We have compared the properties of TANs in WT and CXCR2 KO tumors and observed that CXCR2 KO TANs had a pro-tumor TAN2 profile. In clinical samples, we report that CXCR2 is expressed mainly by neutrophils. Moreover, the levels of CXCR2 are higher in triple negative compared to luminal breast cancers. Interestingly, the higher recruitment of CXCR2 expressing neutrophils is associated with a better prognosis of the patients, suggesting that CXCR2 expressing neutrophils are contributing to the fight against tumor progression. We have analyzed the individual properties of WT and CXCR2 KO neutrophils from mice and we have observed differences in terms of maturation, ROS production, phagocytic ability and transcriptomic profile. This study reinforces the novel potential of tumor associated neutrophils in the control of breast cancer progression. Moreover, we highlight the particular role of CXCR2 in neutrophil function.

Cancer-associated fibroblast spatial heterogeneity and EMILIN1 expression in the tumor microenvironment modulate TGF-β activity and CD8+ T-cell infiltration in breast cancer

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The tumor microenvironment (TME) and its multifaceted interactions with cancer cells are major targets for cancer treatment. Single-cell technologies have brought major insights into the TME, but the resulting complexity often precludes conclusions on function. Therefore, we combined single-cell RNA sequencing and spatial transcriptomic data to explore the relationship between different cancer-associated fibroblast (CAF) populations and immune cell exclusion in breast tumors. Our data show for the first time the degree of spatial organization of different CAF populations in breast cancer. We found that IL-iCAFs, Detox-iCAFs, and IFNγ-iCAFs tended to cluster together, while Wound-myCAFs, TGF-β-myCAFs, and ECM-myCAFs formed another group that overlapped with elevated TGF-β signaling. Differential gene expression analysis of areas with CD8+ T-cell infiltration/exclusion within the TGF-β signaling-rich zones identified elastin microfibrillar interface protein 1 (EMILIN1) as a top modulated gene. EMILIN1, a TGF-β inhibitor, was upregulated in IFNγ-iCAFs directly modulating TGF-β immunosuppressive function. Histological analysis of 75 breast cancer samples confirmed that high EMILIN-1 expression in the tumor margins was related to high CD8+ T-cell infiltration, consistent with our spatial gene expression analysis. High EMILIN-1 expression was also associated with better prognosis of patients with breast cancer, underscoring its functional significance for the recruitment of cytotoxic T cells into the tumor area. In conclusion, our data show that correlating TGF-β signaling to a CAF subpopulation is not enough because proteins with TGF-β-modulating activity originating from other CAF subpopulations can alter its activity. Therefore, therapeutic targeting should remain focused on biological processes rather than on specific CAF subtypes.

Modelling cancer associated cachexia

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Cachexia, is an acute involuntary weight loss (>5%), associated with different chronic illnesses, and several cancers (pancreas, colon…). It is a global metabolic syndrome triggering adipose tissue and skeletal muscles atrophy, accounting for 30% of cancer patients' deaths. We have developed Drosophila larvae models of cancer-associated cachexia based on localised Notch-driven wing disc overgrowth which recapitulate the different hallmarks of cachexia. In depth profiling of the tumours and of the affected tissues revealed new aspects of cachexia.

We showed that beside the adipose tissue and muscles, the gut is also affected during cachexia. This is reflected in a general atrophy of the gut, and a change in its cellular composition. In particular atrophied guts have fewer stem cells, suggesting an untimely usage and depletion. Screening for the factors secreted by the cachectic tumours identified a role for the Jak/Stat pathway ligand Upd3 in mediating the atrophy of the gut and gut stem cell depletion.

Our Drosophila models also identified a contribution of the Adipose tissue during the atrophy of muscles, suggesting complex inter-organ exchanges during cachexia.

Identification of leukemic stem cells in a novel model of chronic myeloid leukemia

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4 Equal contribution

The development of tyrosine kinase inhibitors (TKIs) that target activity of the BCR::ABL1 fusion protein has revolutionized the treatment of chronic myeloid leukemia (CML). Nevertheless, the persistence of malignant cells, i.e. leukemic stem cells (LSCs), is thought to lead to relapse in more than 50% of patients who stop TKI treatment. The lack of an appropriate experimental model that recapitulates the chronic phase of CML has hindered the study of such LSCs. To identify and study the LSCs that persist upon TKI treatment of CML, we established a novel model of CML that uses a unique tamoxifen-inducible Cre driver to target the expression of a tetracycline-regulated BCR::ABL1 transgene specifically within a fraction of hematopoietic stem cells in Pdzk1ip1-CreER+ TRE-BCR::ABL1 Rosa26Tomato/rtTA-GFP, hereafter referred to as Cre+ BA+ animals. Induction of Cre activity results in the differential fluorescent labeling of, and thus the distinction between, normal hematopoietic cells (Tomato+ GFP-) and those that express BCR::ABL1 (Tomato+ GFP+). We confirmed that BCR::ABL1 expression was specific to Tomato+ GFP+ cells and was not observed in Tomato+ GFP- or Tomato- GFP- cells. We studied the long-term impact of BCR::ABL1 expression in Cre+ BA+ animals. Importantly, our animals showed 52% survival at 200 days, which is ~3 times longer than the widely used Scl-tTA BCR::ABL1 transgenic model of CML. Complete blood counts from Cre+ BA+ animals showed a progressive increase in the number of both platelets and white blood cells, which peaked at day 120 following induction. The leukocytosis was associated with an increase in the fraction of Tomato+ GFP+ granulocytes, the appearance of cells that resembled blasts, as well as an increase in the expression of BCR::ABL1 from peripheral blood cells. Cre+ BA+ animals also developed splenomegaly that was characterized by an infiltration of myeloid cells and extramedullary hematopoiesis. Despite the initial elevated numbers of platelets and white blood cells, these increased numbers were not maintained beyond 4-5 months, likely due to the onset of myelofibrosis of the bone marrow and subsequently the spleen. Our pilot experiments of TKI treatment of Cre+ BA+ animals confirmed the efficient abrogation of disease parameters including in the number of platelets and white blood cells and the fraction of Tomato+ GFP+ granulocytes detected in the peripheral blood. Characterization of the cell surface phenotype and molecular status of such persistent leukemic cells is currently ongoing. Our results demonstrate that our transgenic model recapitulates key aspects of the human disease, notably the chronic phase of CML, and we believe that this is critical for the identification of relevant therapeutic strategies for the elimination of LSCs that persist upon TKI treatment.

SUMOylation Controls AML Cells Migration Through the Regulation of CD36 Gene Expression

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Acute Myeloid Leukemias (AML), is a group of deadly hemopathies, resulting from a deregulation of hematopoiesis in the bone marrow. Despite recent advances in the characterization and prognosis of AML and the hope raised from new targeted therapies, the relapse rate is high and the overall prognosis remains poor, therefore, therapy improvement is still needed. Our team has previously shown that SUMOylation plays a critical role in the AML response to chemotherapies and differentiation therapies. Thus, targeting SUMOylation constitutes a promising approach in the AML treatment. Recently, our team demonstrated that TAK-981, a first-in-class SUMOylation inhibitor, has a promising anti-leukemic effect both in vitro and in vivo AML models.

Our aim is to understand, at the molecular level, the effect of TAK-981 on AML cells. We performed an RNA-seq analysis on a TAK981-treated AML cell line, U937, and we observed a limited effect on gene expression. Among the few overexpressed genes, we identified the CD36 gene, a fatty acid transporter. We could confirm the induction of CD36 at the mRNA and protein level both in cell lines and patient samples treated with TAK981. In addition, we found that the transcription factor PPARδ is involved in the TAK981-induced CD36 overexpression. Surprisingly, TAK981 treatment did not affect lipid uptake & accumulation, suggesting that CD36 overexpression does not affect lipid metabolism. Instead, we found that TAK-981 increased AML cell migration in transwell assays. CD36 was highly expressed in the migrating cells and its inhibition by a specific antibody blunted TAK981-induced AML cell migration.

In conclusion, we demonstrate that the inhibition of SUMOylation with TAK-981 induces a strong upregulation of CD36 expression, via the activation of PPARδ, which increases AML cell migration. Therefore, CD36 could be considered as a target to improve AML response to TAK-981.

Unraveling the role of Polypyrimidine Tract Binding Protein 1 (PTBP1) in Acute Myeloid Leukemia: at the cross-road between metabolism, proliferation and survival

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Background. Acute myeloid leukemia (AML) is one of the most common and lethal hematological cancers in adults with a high rate of disease relapse upon therapy and a 5-year overall survival rate of only 20 to 40%. Alteration of RNA splicing, an essential process involved in RNA maturation, has been shown to support AML initiation and progression and to be associated with therapy resistance. However, our understanding of how changes in RNA splicing may impact AML biology and clinical outcome is currently very limited.

Polypyrimidine Tract Binding Protein 1 (PTBP1) is a multi-functional RNA-binding protein that plays a key role in the regulation of RNA splicing, translation, stability and localization. Besides being one of the best described splicing factors in developmental biology, PTBP1 is known to regulate the metabolism of glucose by controlling the alternative splicing of the key glycolytic enzyme pyruvate kinase M (PKM). Over-expression of PTBP1 has been linked to tumorigenesis and associated with adverse prognosis in multiple cancer types. In AML, PTBP1 was identified as part of a network of splicing factors associated with poor clinical outcome. However, how PTBP1 expression may affect AML biology, metabolism and response to therapy is currently unclear.

Aim. The aim of this study is to unravel the function of the splicing factor PTBP1 in the context of AML biology and metabolism.

Results. Here we show that PTBP1 silencing results in rapid negative selection of human AML cells in vitro independently of their genetic background. In vivo PTBP1 depletion has a strong anti-leukemic effect associated with induction of apoptosis. PTBP1 knock-down results in an increase of PKM1/PKM2 isoform ratio and changes in the rate of glycolysis, mitochondrial oxidative phosphorylation and redox homeostasis. However, these metabolic changes are heterogeneous among cell lines and are uncoupled from the anti-leukemic effect observed in vivo. RNA-sequencing combined with iCLIP analysis indicate that PTBP1 binds preferentially to intronic regions and its depletion leads to widespread changes in alternative RNA splicing. Pathways enrichment analysis showed that PTBP1 regulates the expression and splicing of genes involved in proliferation, DNA damage repair as well as in various metabolic pathways, spanning from the metabolism of glucose to that of amino acids, lipids and pyrimidines. Our preliminary results suggest that, beyond controlling the balance between anaerobic glycolysis and oxidative phosphorylation through the alternative splicing of PKM, PTBP1 may be a central regulator of AML metabolism orchestrating multiple metabolic pathways to support the energy needs, proliferation and redox homeostasis of AML cells.

Conclusions. Overall, our work shows that the splicing factor PTBP1 is essential for the growth and survival of human AML cells. Our results suggest that PTBP1 may be a key regulator of post-transcriptional gene expression in AML cells linking energy metabolism, cell proliferation and survival and, as such, a promising new molecular vulnerability in this disease.

Posters' List

✴ **Poster 1** - Acquired chemoresistance in Pancreatic Adenocarcinoma: Mechanisms involving a stromal ZBTB family transcription factor – **Hippolyte AUDUREAU**

✴ **Poster 2** - Targeting of folate receptor beta expressing by TAM with vectorized magnetic nanoparticles for anticancer therapies – **Chloé BAZILE**

✴ **Poster 3** - Regulation of the tumor immune microenvironment by colorectal cancers – **Vincent CAVAILLES**

✴ **Poster 4** - Imaging the 3D cellular effects of confinement in oncology: Application in pancreatic cancer – **Romina D'ANGELO**

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P01: Acquired chemoresistance in Pancreatic Adenocarcinoma: Mechanisms involving a stromal ZBTB family transcription factor

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Introduction:
Pancreatic ductal adenocarcinoma (PDAC) remains a lethal disease, mainly due to patient relapse after chemotherapeutic treatment. It's characterized by a rich connective tissue, including a heterogeneous population of cancer-associated fibroblasts (CAFs), key players in chemoresistance. Our project focuses on identifying and characterizing stromal targets that would influence the acquisition of tumor chemoresistance.

Methods:
To achieve this goal, we established a mouse model using patient-derived xenograft mice (PDX, n=8 patients) in which sensitive tumors chronically treated with long-term gemcitabine were rendered resistant. From these two tumor types, multiplex tissue images and RNA sequencing (RNA-seq) data were generated. In vitro experiments were also performed to decipher the molecular mechanisms implicating CAFs in PDAC chemoresistance.

Results:
Following unsupervised bioinformatic analysis of these tumor RNA-seq data, we have identified a stromal transcriptional signature correlated with tumor treatment (Gem) response that includes a transcription factor (TF) of the ZBTB family. Moreover, its expression is predominant in the stroma of tumors that are still sensitive to Gem. These results were validated with multiplex tissue images showing the expression of this TF in CAFs. Knowing its importance in the regulation of cellular identity, we have hypothesized that it regulates the activation of CAFs and ultimately their ability to promote acquired chemoresistance. Using CAFsoverexpressing the TF, we've demonstrated in vitro its ability to reduce basal CAFs activation and to prevent their activation in chemoprotective CAFs. We found that these two phenomena are partly due to the inhibition of thesecretion of the pro-tumorigenic cytokine IL-6. Furthermore, our transcriptional signature describes a strong correlation with tumors from patients with the best survival prognosis (cohort Puleo et al. 2018).

Conclusion:
Our research highlights the critical role of this TF in the development of stroma-mediated chemoresistance. We are optimistic that our findings will lead to the development of stroma-specific drugs to prevent treatment relapse in patients with PDAC.

P02: Targeting of folate receptor beta expressing by TAM with vectorized magnetic nanoparticles for anticancer therapies.

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Tumor-associated macrophages (TAM) are well known as protecting tumor cells against apoptosis, immune attacks and therapies. Elimination of these pro-tumoral TAM remains a challenge in cancer therapies. Several ways of TAM targeting exist, however they are not specific, potentially leading to serious adverse effects.

We produced and patented a monoclonal antibody called 6-25 capable to specifically recognize the nurse-like cells (NLC), TAM of the chronic lymphocytic leukemia (CLL), and TAM from different solid cancers. We showed that the 6-25 antibody recognizes the folate receptor beta (FR) at the surface of these cells and is internalize in these cells without inducing any toxicity. The FR is also expressed by the M2 monocytes-derived macrophages (M2M) but not by the M1 monocytes-derived macrophages (M1M) or other myeloid cells.

The goal of the project is to produce a tool that specifically targets and kills pro-tumoral TAM in the tumor in order to sensitize cancer cells to chemo- or immunotherapies.

In cancer treatment, magnetic hyperthermia represents an emerging approach with promising therapeutic potential. The magnetic hyperthermia induces an increase of the temperature following the localized application of a high frequency alternating magnetic field (AMF) to a tumor containing magnetic nanoparticles (MNP), leading to cell death. Iron oxide MNP are highly biocompatible and non-toxic (rapid degradation with iron cations recycling), which allows their combination with conventional therapies.

Thus, we developed a magnetic nanoparticle based on a PEGylated iron oxide MNP functionalized with the 6-25 mAb (MNP-6-25) as a specific tool to target pro-tumoral TAM expressing the FR or IgG control as a negative control thanks to a Michael reaction, and a fluorophore, the Cyanine 5, allowing its detection.

For this study, two cellular models were used: M2M as expressing FR at their surface, and M1M as negative control without FR at their surface. M2M and M1M were obtained by the culture of monocytes from healthy donors in the presence of appropriate cytokines cocktails.

First, we showed that MNP-6-25 were not toxic toward M1M and M2M in a concentration up to 64 µg Fe_2O_3/mL after 72h incubation. Then, MNP-6-25 binds specifically M2M but not M1M, with a maximum of binding at 48h of incubation at 8 µg/mL. Finally, confocal microscopy imaging showed that MNP-6-25 accumulated in the lysosome of M2M.

Secondly, we performed an alternative model to study the penetration and the specificity of MNP-6-25 in a 2D and 3D co-culture model and in a same time to study the impact of M1M or M2M on the proliferation of cancer cells. We realized 3D co-cultures with M2M or with M1M and A549 (lung cancer cell line) using the technic of ultra-low-attachment plate for the formation of spheroids. We showed that in 2D co-culture there is a higher proliferation with M2M than with M1M. In 3D co-culture model, we observe a proliferation switch for the 3D co-culture with M1M correlated with an increasing of M2M markers on type 1 macrophages.

In perspective, we plan to evaluate the efficacy and the specificity of MNP-6-25 to target and kill M2M in this 3D model upon application of magnetic field and then the in vivo targeting of macrophages in a murine model of non-small cell lung cancer with MNP-6-25.

P03: Regulation of the tumor immune microenvironment by colorectal cancers

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Colorectal cancer (CRC) is the third leading cause of cancer deaths. Various signaling pathways, which regulate intestinal development and differentiation, play important roles in colorectal carcinogenesis. Our published and unpublished works demonstrate that, in the intestinal epithelium, the transcriptional coregulator RIP140 inhibits cell proliferation, regulates lineage differentiation and participates in the control of genomic stability.

Very interestingly, our recent data indicate that RIP140 is also a driver of tumor immune microenvironment (TIME). We first used genetically engineered mouse models, with a specific deletion of the Rip140 and Apc genes in intestinal epithelial cells (RIP/APCKOint mice), to study how the lack of RIP140 expression reshapes the immune ecosystem around tumors in the colon. RNA-sequencing analyses first demonstrated a higher level of lymphocytes in the TIME of RIP/APCKOint mice, including B and regulatory T cells (Treg). Using immunohistochemistry and mass cytometry imaging, we observed that deletion of the RIP140 gene increases the number of proliferative TLS, as well as their infiltration by Treg. Moreover, RIP/APCKOint mice appeared to respond better to anti-PD1 treatment than wild-type littermates.

We also started to investigate the correlation between the composition of the TIME in human biopsies of CRCs with microsatellite instability and the presence of a mutated form of RIP140, i.e. the RIPMSI dominant negative mutant that we recently identified in 20% of these tumors. Our preliminary data confirmed that the expression of RIPMSI correlates with a shift in the TIME composition and with variations in the level of various cytokines. Altogether, RIP140 appears as a key regulator of major signaling pathways in colon cancer cell with multiple intrinsic effects controlling cell proliferation and genome stability, but also with tumor extrinsic effects which reshape the TIME. The combination of these two types of effects conferes to RIP140 a potential use as a marker of patient survival and response to immune therapies.

P04: Imaging the 3D cellular effects of confinement in oncology: Application in pancreatic cancer

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Context

Intratumoural spatial distribution of mechanical constraints at tissular level and its importance to influence cancer progression are poorly understood. This was explored in murine in situ pancreatic tumours induced by KRAS and TP53 mutations, using shear wave elastography (SWE). SWE reproducibly categorized tumours with selective mechanical parameters. Tumours with low heterogeneity of rigid spots were associated with worse cancer-induced lethality. Interestingly, we found that SWE high heterogeneity in part estimates increased compressive constraints. Compressive constraints emerge from confinement. To study the underlying mechanisms, we are modelling this heterogeneity of confinement in ex-vivo 3D cultures.

Methods

Pancreatic cancer cells were grown in 3D at different time making size of spheroids from 200µm to 500µm. Confinement is mimicked through embedding in hydrogels. Main studied parameters are cadherin expression and/or localisation, fibrillar actin and proliferative cells capacities. 3D imaging is performed on cleared spheroids using different methods adapted to the size.

Results

Here we will show some tools to improve immunolabeling quality, clearing efficiency as well as the choice of the type of microscopy on the whole mount spheroids. The sample specificity (pancreatic tumour cell line known to spontaneously secrete matrix components) have to be taken into account for the clearing strategies.
We will illustrate results comparing different clearing methods and the relevance of matching Refractive Index combined with different microscopies approaches (confocal, spinning disk with different disk geometry).

Conclusions

High resolution 3D imaging allow deep analysis of subcellular structures and identify those that are most significantly implicated in mechanosensing. These technological approaches will be used for understanding how confinement affects cancer cell aggregation and cytoskeletal organisation in pancreatic cancer spheroids.

References

_Rizzuti I. et al., Guillermet-Guibert J,* Delarue M*. *Physical Review Letter 2020*
_D'Angelo R., Ligat L. Optimization of 3D High Content Screening (HCS) acquisitions on thick samples, *Workshop 083, MiFoBio 2023*
_D'Angelo et al., Kit du débutant en Transparisationhttps://rtmfm.cnrs.fr/gt/gt-transparisation/kit-du-debutant-transparisation/, Groupe de travail en Transparisation, 2019
_D'Angelo et al., Samples clearing guidelines https://rtmfm.cnrs.fr/gt/gt-transparisation/samples-clearing-guidelines/, Groupe de travail en Transparisation, 2023

P05: Uncovering phenotypic heterogeneity of drug tolerance in oncogene-addicted non-small cell lung cancer

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Targeted therapies are effective treatments for advanced lung cancer patients bearing oncogene drivers alterations, but are not curative due to the inevitable apparition of resistance. The resistant proliferative cells may arise from a small population of drug tolerant cells (DTC) through non-genetic reprogramming. Deciphering the vulnerabilities of these cells is therefore essential to propose new therapeutic strategies to patients, but the molecular mechanisms underlying drug tolerance is still poorly understood.

We performed an extensive step-by-step characterisation of the molecular and phenotypic processes involved in the adaptive response to targeted therapies in vitro and in Patient-Derived Xenograft (PDX) models, at the single cell level. We found that the DTC population is a highly dynamic and heterogeneous state, with both stably G1-arrested cells and early cycling escapers, that exhibit contractility features via Rho/ROCK-dependent actin cytoskeleton remodelling. We also shed light on the phenotypic switch that DTC undergo, as they transition to a pseudo-healthy lung phenotype under treatment, while acquiring mesenchymal characteristics as they develop resistance mechanisms. We identified a farnesyltransferase inhibitor compound that efficiently prevents relapse to targeted therapies in various cell lines, and strongly delays the acquisition of resistance in several PDX models. We are now investigating whether our characterisation of drug tolerance is relevant in patients, by following a cohort of 40 EGFR-mutated lung cancer patients treated with targeted therapy. We are using liquid biopsies to monitor circulating tumour DNA and characterise circulating tumour cells during treatment.

P06: Implication of carboxyamidotriazole, an oral inhibitor of non-voltage-dependent calcium channels on glioblastoma stem cells.

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Glioblastoma (GBM) is the most frequent and aggressive brain cancer. The treatment of this cancer is based on radiotherapy, chemotherapy with temolozomide (TMZ) and surgery but the median survival is poor, at around 15 month. The tumor recurs in approximately 90% of patients. This relapse has been assigned to the presence of cancer stems cells. This small subpopulation of tumor cells shares with stem cells, proliferation and self-renewal properties. To improve patient treatment, a multicenter phase IB trial has been done using TMZ and carboxyamidotriazole (CAI), an oral inhibitor of non-voltage-dependent calcium channels. This study showed that the combination of CAI and TMZ is safe, and in recently diagnostic patients, median progression-free survival was up to 28 months. This therapeutic combination showed promising signals of activity in this difficult-to-treat population.

In non-excitable cells like GSC, the main calcium entry is via calcium channels called "store operated channels" (SOC). Previous work of our laboratory showed that the pharmacological inhibition of SOC decreases proliferation, impairs self-renewal, and reduces expression of the stem cell marker SOX2 in GSC. The present study addresses the impact of CAI in GSC calcium homeostasis and in stem cell properties.

In human GSC, CAI decreases store operated channels entries (SOCE) in dose-dependent manner and reduces proliferation and self-renewal. The inhibition of the main store operated channel Orai 1 reduced SOCE and no additional effect was observed when Orai 1 inhibition was combined with CAI, suggesting that CAI acts via SOCE pathways.

So, our results show an effect of CAI on GSC responsible for tumor growth and relapse. Because calcium signaling is involved in regulating tumor progression and stem cells properties and because CAI can be used therapeutically, our works provide a better understanding of the mechanisms of action of CAI and could play a role in improving the therapeutic management of GBM.

P07: Single-cell quantitative phosphoproteomics analysis of cellular heterogeneity to predict melanoma response to MAPK inhibitors

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Advanced or metastatic melanoma, an aggressive form of skin cancer, represents a major challenge in the field of oncology. In 50% of cases, BRAF kinase mutations play a significant role in the initiation and progression of this disease, by impacting the MAPK mitogenic signaling network. The use of BRAF and MEK kinase inhibitors has revolutionized the treatment of BRAF-mutated metastatic melanoma, bringing impressive clinical responses. However, a quarter of patients do not respond to these treatments. What's more, in most responders, due to the great plasticity and heterogeneity of melanoma, tumors eventually develop resistance via a variety of mechanisms, leading to recurrence. These resistance mechanisms almost invariably lead to the reactivation of MAPK and/or PI3K/AKT signalling networks, making these networks central players in the resistance of melanoma to targeted kinase inhibitor therapies. Our hypothesis is that primary and acquired resistance to therapies may be predetermined by biomarkers linked to the initial state of these intracellular molecular networks. In addition, various studies have shown that certain subpopulations of melanoma cells are less sensitive to MAPK inhibitors, and are therefore the reservoir of the persistent cells that constitute pre-recurrent residual disease.Our aim is to analyze the heterogeneity and plasticity of the MAPK signaling network to predict the response of metastatic melanoma to current clinical inhibitors. To this end, we have developed a new technique for quantitative single-cell phosphoproteomic analysis using mass cytometry (CyTOF). We optimized the experimental protocol for adherent cells and to preserve the phosphorylation state of the proteins. We built a panel of antibodies to quantitatively analyze 28 phosphoproteins of signaling networks, 6 markers of melanoma subpopulations and 6 markers of proliferation, apoptosis and cell cycle. Finally, we barcoded the cells for simultaneous analysis of the 451Lu cell line, sensitive or resistant version, disrupted by 7 inhibitors over short or medium timescales. At the scale of overall cell populations, comparison of sensitive and resistant cells shows a change in cell identity accompanied by drastic remodeling of MAPK and PI3K/AKT networks. At the single-cell level, we detected several subpopulations among sensitive cells with MAPK and PI3K/AKT networks distinct from the majority population, demonstrating that these networks and how they are disrupted by inhibitors are heterogeneous at the cellular level. By following inhibitor-treated cells for several days, we noticed an early adaptation of some cells to inhibitors, suggesting their ability to survive as persistent cells thanks to their molecular plasticity. These results are very encouraging and demonstrate the benefits of studying phosphoproteomics at the single-cell level. We now need to associate this molecular heterogeneity with the subpopulations of melanoma cells responsible for residual disease, and determine how they contribute to the acquisition of resistance.

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P08: Role of host autophagy in acute myeloid leukemia metabolism and resistance

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Despite the efficacy of conventional chemotherapies currently used in clinical, relapses are frequent in acute myeloid leukemia (AML) and are caused by the growth of relapse-initiating chemoresistant leukemic cells (RICs). That's why, the prognosis in human AML is poor and survival remains low. Thus, a better understanding of resistance mechanisms is needed to reduce relapse and improve survival in AML patients. AML cells, similar to numerous cancer cells, are able to reprogram their metabolism to provide energy and nutrients for their growth. My laboratory and others demonstrated that RICs have an increased oxidative metabolism and relies on fatty acids and amino acids metabolism to survive to chemotherapy and targeted therapy. Several pathways could sustain this metabolism and especially the mechanism of autophagy. Indeed, autophagy is a catabolic process involved in cancer biology regulation and metabolism. Earlier studies on autophagy and tumor growth mainly focused on tumor autophagy. However, similar to tumor autophagy, host/microenvironment autophagy has also been recently implicated in tumor growth promotion. Even if the molecular mechanisms are not fully understood, studies have shown that host autophagy can supply amino acids as well as other substrates or regulate immune response to promote the growth of melanoma cells, pancreatic cancer cells and Drosophila melanogaster malignant tumor.

However, up to now, no study has investigated the role of host autophagy in AML therapeutic resistance. Given the major role of the microenvironment in therapeutic resistance, we hypothesize that host autophagy may be involved in AML therapeutic resistance through its role as a metabolic regulator. Using innovating in vitro and in vivo models for autophagy deficiency, I wish to 1) validate host autophagy contribution in AML metabolism and therapeutic resistance in vitro and in vivo, 2) identify the molecular mechanisms implicated.

These experiments will help to gain a better understanding of the contribution of host autophagy to AML metabolism and therapeutic resistance mechanisms. Herein, this original project will lead to the identification of new molecular targets whose modulation should disrupt the metabolism of AML, making it possible to restore sensitivity to treatments.

P09: A metabolic crosstalk occurs between human bone marrow adipocytes and prostate cancer cells

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In localized prostate cancer (PCa), we have demonstrated that periprostatic adipocytes increase tumor progression by providing cancer cells with fatty acids (FAs) released after the activation of lipolysis, involving the hydrolysis of triglycerides (TG) [1]. In advanced PCa, the majority of metastases are found within the bone, where tumor cells can interact with bone marrow adipocytes (BMAds). However, whether a metabolic crosstalk between primary BMAds and PCa exists and favors tumor progression remains to be determined. Thanks to a collaboration with orthopedic surgeons, we obtain human bone marrow adipose tissue (BMAT) during hip replacement surgery. There are two types of BM-Ads: those contained in the red BMAT (rBMAds) and those contained in the yellow BMAT (yBMAds), which have been characterized by my team [2]. Since PCa metastatic sites are frequently found in proximity to rBMAds, we established a 3D culture of these adipocytes in a fibrin matrix to preserve their viability for up to 5 days and cultured them with PCa cells.

Under coculture conditions, PCa cells exhibited an increase in neutral lipid content, primarily composed of TG. Using rBMAds loaded with fluorescent FAs, we directly demonstrated that FAs released by rBMAds are taken up by cancer cells and re-esterified into TG. These data provide the first evidence of a metabolic crosstalk between primary human rBMAds and PCa cells. Through lipidomic approaches, we determined that rBMAds release FAs mainly palmitate, oleate, and linoleate. However, like yBMAds [2], we found that rBMAds are devoid of lipolysis due to a profound decrease in the expression of the last two enzymes of the lipolytic pathway. These data suggest that an original mechanism, independent of classical lipolysis, may be involved in the release of FAs by rBMAds. Interestingly, the first lipolytic enzyme, ATGL (Adipose Triglyceride Lipase), and its cofactor are expressed in rBMAds and could participate in the release of FAs through an unusual incomplete lipolytic process which is currently under investigation. Once inside tumor cells, we found that FAs are stored as TG but are also oxidized in mitochondria. However, this increased fatty acid oxidation is not associated with increased ATP production. Thus, FAs taken up by PCa cells are not primarily used for energy production but could be involved in other processes, such as transcriptomic remodeling. RNASeq and gene ontology analyses of PCa cells cocultivated with or without rBMAds reveal clear differences in migration pathways. This process is a key step in the propagation of cancer cells from one bone metastatic site to other bone metastatic site, making the disease highly aggressive. Functional experiments confirmed that rBMAds specifically increase the migratory capacity of different PCa cell lines without any increase in proliferation. Whether this pro-migratory effect of rBMAds is due to the transfer of FAs is under investigation.

In conclusion, the metabolic crosstalk between rBMAds and PCa cells could contribute to the propagation of bone metastasis. Deciphering this crosstalk, including other metabolites, could lead to pharmacological targets for the treatment of bone metastases, for which therapeutic options remain very limited.

[1] Laurent V, Toulet A, Attané C, et al. Periprostatic Adipose Tissue Favors Prostate Cancer Cell Invasion in an Obesity-Dependent Manner: Role of Oxidative Stress. Mol Cancer Res. 2019;17(3):821-835. doi:10.1158/1541-7786.MCR-18-0748
[2] Attané C, Estève D, Chaoui K, et al. Human Bone Marrow Is Comprised of Adipocytes with Specific Lipid Metabolism. Cell Rep. 2020;30(4):949-958.e6. doi:10.1016/j.celrep.2019.12.089

P10: Vicious circle between tumor and pancreatic adjacent tissue: the implication of solid stress

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by an abundant stroma composed predominantly of activated fibroblasts (CAFs) and dense extracellular matrix (ECM). Tumor cell growth and ECM modifications generate forces named "solid stress" documented to promote tumor progression. While it is recognized that these forces also result in compression of surrounding healthy tissue, their impact on the biology of adjacent normal cells, especially normal fibroblasts (Pancreatic Stellate Cells, PSCs) remain poorly described.

In order to mimic the compressive force transmitted by the tumor to the surrounding healthy tissue, we added an agarose cushion on a monolayer of PSCs isolated from human healthy pancreas. We show that a low pressure (85 Pa) induces a strong expression of at least three of the main markers of activated fibroblasts. This phenomenon is associated with a reorganization of smooth muscle actin into stress fibers associated with drastic changes in cell morphology, mitochondrial network rearrangement, and the activation of the mechanosensor FAK. We identified that Akt and ERK are two main pathways involved. The consequence of this mechano-induced fibroblast activation is an important increase of their ECM production and secretion. Finally, we show that the ECM secreted by the "mechanically activated fibroblasts" induce epithelial-mesenchymal transition of tumor cells.

We are currently developing an original 3D device, allowing the application of a quantifiable and homogeneous pressure on cells embedded in a gelatin methacrylate (GelMA) hydrogel, to verify our results in a context more relevant to the pathology. This device, compatible with a wide range of techniques (IF, WB, RNAseq, live imaging…) will enable us to deeply characterize the impact of tumor-generated pressure on normal adjacent pancreatic fibroblasts cultivated alone or in co-culture with tumor cells.

In conclusion, we obtained the proof of concept in 2D, and will verify it in 3D, that solid stress activates PSCs in a durable manner and confer them protumoral properties that most likely will support and favor tumoral progression.

P11: Periprostatic adipose tissue: a new source of androgens for castration resistant prostate cancer

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Androgen deprivation is the treatment of choice for locally advanced prostate cancer (PCa) and involves the inhibition of testosterone secretion by the testes. However, the disease will progress into castration resistant PCa (CRPC) after a few years. An emerging mechanism of CRPC involves the ability of cells to find other sources of androgens in the tumour microenvironment in order to produce active forms of androgens such as 5α-dihydrotestosterone (5α-DHT), as recently shown for gut microbiota and cancer-associated fibroblasts.

The aim of our study was to determine if periprostatic adipose tissue (PPAT), an adipose depot surrounding the prostate has a specific steroid metabolism that could favour CRPC occurrence. We characterized the sex steroid content and the steroidogenic enzyme expression profile of human PPAT and abdominopelvic adipose tissue (APAT, as control) from patients with PCa who underwent a radical prostatectomy, by Gas Chromatography-Mass Spectrometry (GC-MS) and RT-qPCR. The effects of PPAT/APAT-conditioned media (CM) on PCa cell growth and proliferation were evaluated by real-time imaging system Incucyte and Brdu incorporation.

We demonstrated that PPAT contains more of certain steroid metabolites such as 5α-androstanedione and epiandrosterone compared to APAT, involved in the alternate routes for 5α-DHT synthesis in CRPC. Within PPAT, mature adipocytes appear to be the main source of androgen metabolites. The regulation of the expression of steroidogenic enzymes could explain the higher content of 5α-androstanedione and epiandrosterone in PPAT. Furthermore, we demonstrated that PPAT-CM can support cell growth and proliferation of an androgen-dependent cell line LNCaP cultivated in androgen-deprived medium but not in androgen containing medium. This effect is inhibited by an androgen receptor (AR) antagonist, bicalutamide, and PPAT-CM upregulates the expression of AR target genes (KLK3 and FKBP5). Finally, PPAT-CM reverses the acute regulation of certain steroidogenic enzymes induced in the absence of androgens. Altogether, these results bring arguments in favour of a functional effect of the steroid metabolites contained in PPAT on PCa cells and demonstrate that PPAT could represent a new source of androgens for CRPC.

P12: Role of proteins involved in epithelial integrity and polarity, regulated by de(phosphorylation) by Syk or PTPN13, in mammary tumor invasion

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The kinase Syk (Spleen tyrosine kinase) and phosphatase PTPN13 (tyrosine-protein phosphatase non-receptor type 13) behave as tumor and metastasis suppressors in breast cancer. The French host laboratory also demonstrated a role for Syk and PTPN13 in cell junction stabilization. However, the downstream signaling effectors of Syk and PTPN13 remain largely unknown. Using phosphoproteomics and interactomics approaches, the team identified ARHGEF7 (Rho guanine nucleotide exchange factor 7) and PARD3 (Par-3 family cell polarity regulator) as common putative effectors of Syk and PTPN13. In this study, we aim to investigate whether these proteins are direct partners or substrates of Syk and PTPN13. Their function, consequences of their (de)phosphorylation and role in epithelial integrity in 2D/3D models will be studied. Finally, we will characterize the proteins of interest in genetically engineered mouse models and based on the obtained results, clinical studies will be performed on breast cancer biopsies.

We showed that PARD3 and ARHGEF7 co-immunoprecipitate with Syk and PTPN13. Immunofluorescence analysis on human breast cancer cells also revealed the colocalization of Syk and PTPN13 with PARD3 and ARHGEF7 at cell junctions.

This study will characterize new signaling effectors through which Syk and PTPN13 exert their anti-proliferative and anti-invasive effects in epithelial cells, which may aid in the identification of novel biomarkers and therapeutic targets.

P13: Role of MAPK signaling in T-cell mediated anti-tumor immune responses

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Tumorigenic activation of Mitogen-Activated Protein Kinases (MAPKs) is central for translational reprogramming of cancer cells, as it allows rapid cell adaptation to highly dynamic environment by promoting selective mRNA translation. Amongst these MAPKs, we found that alterations of the MEK kinase MAP3K8 (also known as TLP-2/COT) constitute an alternative to BRAF mutations. Accumulation of MAP3K8 protein correlates with MEK/ERK activation and poor patient outcome in high-grade serous ovarian cancers (HGSOC) which are rarely mutated for BRAF. By combining analysis in HGSOC cohorts of patients and relevant cellular and mouse models, we found that constitutive activation of MAP3K8/MEK promotes tumour growth and confers a new translational landscape by regulating the assembly and activity of the translation initiation complex eIF4F. In addition, first analyses show that MAP3K8 expression by tumour-infiltrating T lymphocytes (TILs) suppresses anti-tumoral responses. Proliferation and activation of TILs increases locally in the tumours upon Map3k8 deletion suggesting that MAP3K8 might restrain T cell activation after cancer cell priming by controlling translation of selected mRNA targets. By using knock out (KO) mouse models, we are now uncovering the intrinsic function of MAP3K8 in TILs and assess changes in the MAPK pathway and mRNA translational programs involved in cancer progression. Deciphering the crosstalk of cancer cells and TILs at the level of mRNA translation will be the basis of future combination therapies enhancing immune checkpoint inhibitor efficacy by targeting MAP3K8.

P14: Trogocytosis of cancer-associated fibroblasts promotes pancreatic cancer growth and immune suppression via phospholipid scramblase anoctamin 6 (ANO6)

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The fibroblastic stroma comprises most of pancreatic adenocarcinoma mass and is remarkably devoid of functional blood vessels leaving an unresolved question of how pancreatic cancer cells obtain their essential metabolites and especially water-insoluble lipids. Contrary to the previously held assumption that cancer cells uptake lipids directly from the interstitial fluid, we have found a critical role for cancer-associated fibroblasts (CAFs) to obtain and transfer blood-borne lipid particles to cancer cells via trogocytosis, a process of "nibbling" of plasma membranes between two cells engaged in synapse-like membrane contacts. Whereas trogocytosis has been described in normal development, the biochemical and signaling regulators of trogocytosis between CAFs and PDAC cells have not been defined.

We determined that CAF membrane trogocytosis is triggered by externalized phosphatidylserine (PtdSer), and blockade of PtdSer in vitro transiently deters trogocytic uptake of CAF membranes. We have also discovered a phospholipid scramblase anoctamin 6 (ANO6) expressed in CAFs as the essential trogocytosis regulator to promote cancer cell survival. Mechanistically, CAF-cancer cell membrane contacts induce cytosolic calcium influx via Orai channels, which activates ANO6 and results in phosphatidylserine exposure on CAFs. As a promising therapy target, ANO6 protein is highly expressed in PDAC tumor mass in cancer cells, endothelial cells and CAFs and is a negative prognostic biomarker for survival. Depletion of ANO6 in co-implanted CAFs dramatically reduced the growth of orthotopic pancreatic tumor grafts. Furthermore, pharmacologic inhibitors of ANO6 with clinically available antibiotics niclosamide or clofazimine potently blocked cholesterol uptake in vivo by PDAC cells.

Our findings indicate a novel trogocytosis function for CAFs in highly desmoplastic carcinomas as the main mechanism of lipids delivery to cancer cells. CAFs do so by expressing PtdSer as "eat me" signals. This process is regulated by Ca2+-dependent phospholipid scramblase ANO6. Re-purposing of clinically available ANO6 inhibitors could make a tangible impact on treatment of PDAC patients in the near term.

P15: Targeting the YAP1/TAZ pathway in gastrointestinal stromal tumors (GIST)

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Gastrointestinal stromal tumors (GIST) are the most common sarcoma of the gastrointestinal tract. These tumors mostly arise from oncogenic gain of function mutations in receptor tyrosine kinase genes, mainly *KIT*. Current treatments are based on tyrosine kinase inhibitors, such as imatinib mesylate (IM). However, a substantial proportion of patients develop over time resistance to such therapy and cancer relapse occurs rapidly [1]. One cause of IM resistance is the activation of signaling pathways unsensitive to IM among which the YAP1/TAZ pathway is a promising candidate [2,3]. The Hippo pathway effectors YAP1 and TAZ are master regulators for multiple cellular processes and cancer development. Dissecting YAP1/TAZ pathways and elucidating down-stream effects could help to better understand GIST resistance to conventional therapy and to develop novel therapeutics.

Thus, we propose a new therapeutic approach targeting YAP1/TAZ in GIST. The objective is to manipulate YAP1/TAZ expression using specific siRNAs in order to assess their impact on IM sensitive or resistant GIST cells. For this purpose, we have designed and validated siRNAs efficient in specifically targeting YAP1 or TAZ protein expression individually. For the cellular internalization, siRNAs were transfected with our peptide-based nanoparticles (WRAP) [4].

First results evidenced that WRAP nanoparticles encapsulating siYAP1 or siTAZ could specifically reduce YAP1 or TAZ levels up to 70% in GIST cells. More importantly, we determined the efficient ratio to obtain nanoparticles simultaneously encapsulating both siRNAs (siYAP1/siTAZ) to silence both proteins to evaluate a potent additive effect. The cellular consequences of this YAP1/TAZ inhibition will be further investigated regarding the KIT activity, the YAP1/TAZ downstream pathways, viability, proliferation and apoptosis of IM sensitive/resistant GIST cells.

In conclusion, reducing YAP1/TAZ activity in GIST should allow to reduce IM doses and/or to re-sensitize cancer cells to IM treatment.

References

[1] Schaefer, I.-M., DeMatteo, R.P., & Serrano, C. (2022). The GIST of Advances in Treatment of Advanced Gastrointestinal Stromal Tumor. American Society of Clinical Oncology Educational Book.

[2] Guérin, A., Martire, D., Trenquier, E., et al. (2020). LIX1 regulates YAP activity and controls gastrointestinal cancer cell plasticity. Journal of Cellular and Molecular Medicine.

[3] Ruiz-Demoulin, S., Trenquier, E., Dekkar, S., et al. (2023). LIX1 Controls MAPK Signaling Reactivation and Contributes to GIST-T1 Cell Resistance to Imatinib. International Journal of Molecular Sciences.

[4] Konate, K., Dussot, M., Aldrian, G., et al. (2019). Peptide-Based Nanoparticles to Rapidly and Efficiently "Wrap 'n Roll" siRNA into Cells. Bioconjugate Chemistry.

P16: Metabolic crosstalk between mammary adipocytes and tumor cells: role in the aggressiveness of a specific metabolic subtype of triple-negative breast cancers

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Among breast cancers, triple-negative breast cancer (TNBC) remains an unmet medical challenge due to its aggressiveness and the absence of targeted therapies. Recently, TNBC samples have been classified into three heterogeneous metabolic-pathway-based subtypes (MPSs) with distinct metabolic features (Gong *et al. Cell Metabolism,* 2021). The group with the worst prognosis (MPS2) exhibits an increased capacity to uptake exogenous free fatty acids (FFA). Our hypothesis is that the interaction of these cells with tumor-surrounding mammary adipocytes (the main source of FFA in the tumor microenvironment (Attané *et al. Trends in Cancer,* 2020)) is a key event in their aggressiveness.

We used a recently described model of co-culture, established by our team (Rebeaud *et al. Scientific Reports* 2023), between human isolated mammary adipocytes (M-Ad) grown in 3D matrices and human cell lines representative of the 3 MPSs to investigate: i) the transfer of lipids between M-Ad and the different MPS tumor cells using immunofluorescence and lipidomic approaches ; ii) the consequences of this lipid transfer on metabolic remodeling (fatty acid oxidation (FAO) activity, Seahorse) and iii) tumor aggressiveness, including survival, proliferation, and chemoresistance, using real-time imaging.

When co-cultivated with adipocytes, the MPS2 group exhibits a higher accumulation of lipids (about 10-fold) than the other two groups, confirming our main hypothesis. The three MPS groups induce lipolysis in M-Ads at comparable levels, suggesting that the overexpression of FFA transporters is the cause of the higher lipid accumulation in MPS2 cells. In fact, we found that some transmembrane and intracellular lipid transporters (FATP3, FATP4, and FABP5) are overexpressed in MPS2 cells, and their function is being investigated using pharmacological and siRNA approaches. This transfer of lipids into the MPS2 cells leads to a metabolic remodeling favoring an increase in FAO activity, uncoupled from mitochondrial respiration and ATP production. Interestingly, MPS2 cells overexpress both CPT1a, involved in the transport of FFAs into mitochondria, and enzymes involved in FAO. This lipid accumulation and metabolic remodeling in co-cultivated MPS2 cells lead to increased survival (but not proliferation) and selective resistance to chemotherapeutic agents that induce oxidative stress and ferroptosis (e.g., doxorubicin). This effect is replicated by exposing MPS2 cells (but not the other MPSs) to oleate, a monounsaturated fatty acid (MUFA).

In conclusion, we have demonstrated that M-Ads specifically induce increased survival and chemoresistance in MPS2 cells. Our current hypothesis is that both reduced oxidative stress related to uncoupled FAO and changes in the balance between mono- and polyunsaturated FAs (in favor of MUFA) reduce ferroptosis, which is responsible for the observed phenotypical changes. Identifying key steps in this process, such as the involved lipid transporters, could lead to the identification of risk stratification markers and new pharmacological targets in these aggressive diseases.

P17: MAPK pathway-regulated function of RBM34 in the nucleolar stages of the synthesis of the human 60S ribosomal subunit

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Ribosome biogenesis involves the synthesis by RNA polymerases I and III of ribosomal RNA (rRNA) precursors, their packaging into precursor particles (pre-ribosomes), their modification, processing and association with ribosomal proteins (RPs) to generate the mature ribosomal subunits. This process requires scores of assembly factors (AFs) that transiently interact with pre-ribosomes to fulfill specific functions in the maturation process. Signal transduction cascades tightly regulate this process to adapt ribosome biogenesis to cell growth requirements. Alterations in these signalling pathways promote unrestrained ribosome production and increased global protein synthesis that are critical features of cancer initiation and progression. The MAPK pathway regulates ribosome production at different stages, including transcription by RNA polymerases I and III and translation of ribosomal proteins (RPs). Our recent data suggest that the MAPK pathway also regulates the co- and post-transcriptional stages of ribosome synthesis, to coordinate the synthesis of the primary transcripts, their packaging into pre-ribosomes and the whole maturation process. In this work, we are addressing the role of RSK kinases, the downstream effectors of the MAPK signalling pathway, in the maturation of pre-60S particles, the precursors to the large ribosomal subunits. We have identified RBM34, a protein involved in the early, nucleolar stages of this process as a RSK substrate and showed that some phosphorylation events of RBM34 are under the control of the MAPK pathway. We are now investigating the function of RBM34 in the maturation of pre-60S particles and the impact of its regulation by the MAPK pathway in both normal and cancer cell lines. In parallel, we have undertaken a screen to identify exhaustively nucleolar RSK targets in melanoma cells using a cell fractionation technique combined with large-scale phospho-proteomic analyses. This work will lead to a comprehensive view of the impact of the MAPK signaling pathways on the co- and post-transcriptional stages of ribosome biogenesis in both normal and cancer cells.

P18: Paracrine interactions between Cancer-associated fibroblasts (CAFs) and colorectal Circulating Tumor Cells (CTCs)

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Circulating tumor cells (CTCs) are tumor cells that shed from the primary tumor and intravasate into the peripheral blood circulation system in order to further invade distant organs to form metastasis. CTCs have a strong stemness phenotype: self-renewal, plasticity and tumor initiation ability. Previous studies have demonstrated that CTCs do not travel in the bloodstream alone, but rather are accompanied by clusters of immune or stromal cells such as platelets cells or cancer associated fibroblasts (CAFs). CAFs are complex and abundant within the tumor microenvironment involved in the growth, invasion, and chemoresistance of cancer cells. This collective migration unit enhances tumor cell survival and colonization in distant organs. The aim of the present study is to decipher the paracrine interactions between CAFs and colorectal CTCs in vitro. To do so, a conditioned medium (CM) has been collected following a 48hrs co-culture of CAFs and colorectal cancer patient derived CTCs. The CM significantly improves CTC viability and proliferation. An increase in the self-renewal capacity was also observed, accompanied by a decrease of stem cell marker expression/activity (ALDH1 and CD44v6). Taken together, these results suggest that the role of CAFs, present in the blood circulation, is to induce CTC differentiation from stem cell to progenitor phenotype in order to increase their proliferative capacity to prepare them to form metastasis. To validate this hypothesis, we are currently testing successive passages of CTCs in the presence of CM. Only stem cells can be maintained indefinitely in 3D culture, whereas progenitor cells would be depleted. This, in vitro, project will allow us to evaluate the role of paracrine interactions between CAFs and CTCs in the formation of metastasis. Finally, a secretome study is ongoing to determine novel secreted proteins involved in CTC transformation in presence of the CM to better trigger CTCs.

P19: Small ORF encoded peptides as a novel source of tumorigenesis and cancer cachexia regulators

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Tissue homeostasis relies on the balance between cell proliferation and differentiation, which disruption can lead to hyperplasia and tumor formation. In some cases, the tumor can produce deleterious signals that induce the wasting of peripheric organs that leads to a significant weight loss. This organ loss syndrome, known as cancer cachexia, is a multifactorial metabolic disorder that represents a major morbidity factor. It is observed in over half of all cancer patients, with a higher prevalence in males. This syndrome of muscle and/or fat weight loss is unfortunately difficult to reverse. As the mechanisms involved in the onset of cachexia are still poorly understood, our aim is to identify new diffusible molecules involved in the onset and persistence of cachexia from the family of small ORF encoded peptides (smORF peptides), also known as microproteins. Until recently, small ORFs (less than 100 codons) were considered non-coding in order to avoid the annotation of millions of false ORFs. However, the improvement and the development of bioinformatics, mass spectrometry and ribosome profiling have made it possible to annotate hundreds, if not thousands, of novel smORFs encoding for putative smORF peptides in all species. As these smORFs, present in both coding and non-coding RNAs, remained ignored for a long time, they represent a reservoir of potential new bioactive molecules. Using Drosophila as an integrated animal model, we induced intestinal cancer that causes peripheral organ wasting mimicking cancer cachexia. We generated transcriptomes of tumorigenic intestines and cachexic organs in males and females. A bioinformatics study identified annotated and putative smORF peptides differentially expressed according to tissue and sex. To define an exhaustive list of translated smORF during disease, presents in non-coding and coding RNAs, which remains difficult to predict with bioinformatics and machine learning methods, we are performing ribosome-profiling. We are currently investigating the role of a novel secreted peptide, specific to female cachexic organs, to elucidate its role in the establishment of cachexia. Our project will highlight the smORF peptides as a new source of tumor formation regulators and inter-organ communication actors leading to cachexia.

P20: Trogocytosis-mediated transfer of a functional folate receptor beta from Nurse-like cells to Chronic Lymphocytic Leukemia B cells is associated with their activation status

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Chronic lymphocytic leukemia (CLL) is the most common B cell malignancy in the Western world and is characterized by an accumulation of monoclonal CD5+, mature-appearing cancer B cells in lymphoid tissues and peripheral blood. CLL B cells have been shown to be highly dependent on the tumor microenvironment (TME), required for their survival and proliferation. The TME is mostly located in the secondary lymphoid organs, - lymph nodes (LNs) and spleen, but also in the bone-marrow. On the example of LN, where the proliferation of cancer cells occurs (in so-called Proliferation Centers), CLL B cells encounter TME forming cells, including T cells, stromal cells, and Nurse-like cells (NLCs) – the CLL-specific tumor-associated macrophages, expressing CD68, CD163 and CD206. NLCs have been widely shown to play a critical role in the cancer cell survival, homing, proliferation and chemoresistance. Their multifaceted pro-tumoral functionality is essentially mediated by a cell-cell contact, but also a release of various soluble factors. NLCs can be obtained in vitro as a result of long-term CLL PBMC culture (CLL B cells drive differentiation of NLCs from monocytes), facilitating study of these cells. We discovered that NLCs developed in vitro express high level of folate receptor β (FRβ), and that its expression on NLCs correlates strongly with survival of cancer cells. FRβ is efficient at capturing folic acid and is exclusively expressed by a subset of myeloid cells, especially M2-like macrophages. The aim of this study was to decipher the role of FRβ in the interaction between NLCs and CLL B cells. The aim of this work was to study the role of FRβ - the specific marker of protective NLCs, in the interaction between CLL B cells and NLCs.
Interestingly, following the long-term cultures of CLL PBMC we observed an appearance of FRβ+ subpopulation of CLL B cells. It turned out that cancer cells can actively acquire a functional FRβ from NLCs by the process of trogocytosis, facilitating in turn acquisition of folic acid. The frequency of this phenomenon correlates positively with the number of NLCs, but also IL-15 + CD40L activation of CLL B cell. Both cell activation and uptake of essential nutrients (like folic acid) can further push proliferation and survival of cancer cells. Moreover, IHC staining of LNs from CLL patients confirmed a high expression of FRβ by NLCs, and presence of this protein on some CLL B cells in vivo. These results prove the presence of a novel type of interaction between these two-cell types in physiopathology of CLL. Finally, FRβ+ represent an attractive choice for targeted therapies, thanks to its discrete expression, allowing further improvement in CLL management.

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