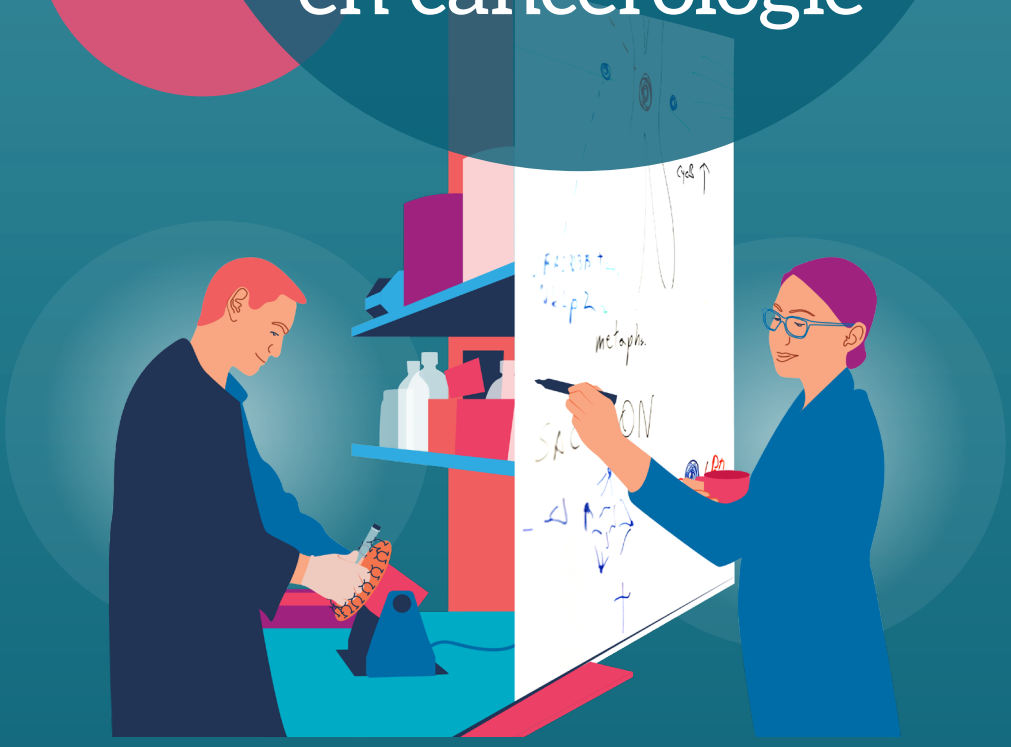
Forum
FONDATION ARC POUR LA RECHERCHE SUR LE CANCER
Reconnue d'utilité publique
Talents Scientifiques

Livret scientifique

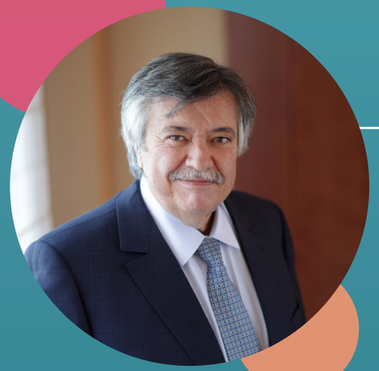
30 novembre 2020

JOURNÉES JEUNES CHERCHEURS en cancérologie



FONDATION ARC POUR LA RECHERCHE SUR LE CANCER
Reconnue d'utilité publique

ÉDITORIAL



CLAUDE TENDIL

Président de la Fondation ARC pour la recherche sur le cancer

Chaque année, la Fondation ARC accompagne plus de 120 d'entre vous, jeunes chercheurs, médecins et pharmaciens, pour vous aider à développer les talents nécessaires à notre combat collectif contre le cancer. Face aux nombreux défis qui se présentent à nous, votre énergie, votre rigueur, votre imagination et surtout votre capacité à réfléchir ensemble, au-delà des frontières strictes de vos domaines de recherche respectifs, sont nos meilleurs atouts. Vous représentez l'espoir de la lutte contre le cancer, et la Fondation ARC est plus que jamais engagée à vos côtés !

PROFESSEUR ÉRIC SOLARY

Président du Conseil Scientifique de la Fondation ARC
Président du jury Hélène Starck 2020
Professeur des Universités, Practicien hospitalier en hématologie

La Fondation ARC est mobilisée depuis sa création pour former les talents qui renouvellent et enrichissent la recherche sur le cancer. Nous vous accompagnons dans l'appréhension des concepts et la maîtrise des technologies. Nous soutenons, lorsqu'elles sont nécessaires, les approches transversales et multidisciplinaires.Pour gagner avec nous la bataille engagée contre les cancers, vous explorez, vous déchiffrez, vous inventez. A vous d'optimiser l'usage des traitements d'aujourd'hui. A vous de préparer la prochaine révolution thérapeutique en cancérologie. Que vous ayez choisi une approche fondamentale ou plus appliquée, nous croyons en vos capacités à modifier l'histoire naturelle de ces maladies dévastatrices, dans l'intérêt des patients, aujourd'hui et demain.

JURYS DES PRIX

HÉLÈNE STARCK ET KERNER 2020

JURY HÉLÈNE STARCK 2020

ÉRIC SOLARY
Président du Jury

PAOLA ARIMONDO, Institut Pasteur, Paris
MARIANNE BURBAGE, Institut Curie, Paris - Lauréate du Prix Hélène Starck 2019
JOSEPH CICCOLINI, Centre de Recherche en Cancérologie de Marseille
BERNARD DE MASSY, Institut de Génétique Humaine, Montpellier
MARIE-CAROLINE DIEU-NOSJEAN, Centre d'Immunologie et de Maladies Infectieuses, Paris
ROBERT FEIL, Institut de Génétique Moléculaire de Montpellier
MJCHAELA FONTENAY, Institut Cochin, Paris
GÉRALDINE GENTRIC, Institut Curie, Paris - Lauréate du Prix Hélène Starck 2018
LIONEL LARUE, Institut Curie, Orsay
GILBERT LENOIR, Vice-président de la Fondation ARC pour la recherche sur le cancer
JULIE PANNEQUIN, Institut de Génétique Humaine, Montpellier
NICOLAS PENEL, Centre Oscar Lambret, Lille
CLAUDE PRIGENT, Institut de Génétique et Développement de Rennes

JURY KERNER 2020

BRIGITTE BLOND, Santé Magazine, Femme Actuelle, Ouest France
CHARLINE DELAFONTAINE, Pleine Vie
KARELLE GOUTORBE, Le Quotidien du Médecin
ISABELLE GRILLOT, Science & Vie TV
SUZY JOURDAN, Côté Santé
SANDRINE MOUCHET, Rose Magazine
ALINE PERRAUDIN, Santé Magazine
FABIENNE RIGAL, Femme Actuelle
AFSANÉ SABOUHI, Ça m'intéresse
JULIE WIERZBICKI, Pharmaceutiques

SOMMAIRE

PROGRAMME

• 8H30 – 8H50 // ACCUEIL DES PARTICIPANTS : CONNEXION AU SITE WEB

• 8H50 – 9H00 // INTRODUCTION DE LA JOURNÉE PAR

Éric SOLARY, Président du Conseil scientifique de la Fondation ARC et Président du Jury Hélène Starck
& Nancy ABOU ZEID, Directrice Scientifique de la Fondation ARC

• 9H00 – 11H00 // SESSION 1 – PRIX HÉLÈNE STARCK ORAL, CATÉGORIE THÈSE

CASANOVA Alexandre
Institut pour l'Avancée des Biosciences, La Tronche
BCAR3 lysine methylation links SMYD2 signaling to breast cancer progression and metastasis

CLUZET Victoria
Université Paris Diderot
Granulosa cell tumors of the ovary: is SIRT1 a new therapeutic target ?

FARGE Thomas
Centre de Recherche en Cancérologie de Toulouse
CD36 promotes extramedullary dissemination and relapse in Acute Myeloid Leukemia

GHOROGHI Shima
Université de Strasbourg
Étude du role des GTPases Ral dans la sécrétion d'exosomes et la formation de metastases

HERVÉ Solène
Institut Curie, Paris
CENP-A chromatin prevents replication stress at centromeres to avoid structural aneuploidy

JACOBS Kathryn
Centre de Recherche en Cancérologie et Immunologie de Nantes Angers
Paracaspase MALT1 regulates glioma cell survival by controlling endo-lysosome homeostasis

JULIEN Manon
Institut de Biologie Intégrative de la Cellule, Gif-sur-Yvette
Deciphering new molecular functions of the BReast Cancer protein 2 (BRCA2): role of phosphorylations during mitosis

LIGIER Maud
Centre de Recherche en Cancérologie de Lyon
Role of ZEB1-mediated cancer cell plasticity in immune escape and resistance to treatments of melanomas

• 11H10 – 11H20 // PAUSE

• 11H20 – 12H50 // SESSION 2 – PRIX HÉLÈNE STARCK ORAL, CATÉGORIE POST-DOCTORAT

BRUNEL Benjamin
Université de Reims Champagne-Ardenne
Raman imaging for clinical histopathology: predicting colon cancer therapies response using spectroscopic markers

GORVEL Laurent
Centre de Recherche en Cancérologie de Marseille
Déterminer l'importance de l'axe TIGIT-PVR dans le développement d'Immunothérapies dans les cancers du col de l'utérus

KRAMARZ Karol
Institute Curie, Orsay
Processing of replication forks at the nuclear periphery: Mechanism and impact on genome stability

MOINDJIE Hadia
Gustave Roussy, Villejuif
Targeting cell metabolism to overcome taxane-based chemoresistance in high-grade serous ovarian cancer

PEGLION Florent
Institut Pasteur, Paris
Role of PTEN loss in Glioblastoma Invasion

SMITH Rebecca
Institut de Génétique et Développement de Rennes
The chromatin remodeler ALC1 underlies resistance to PARP inhibitor treatment

• 12H50 – 14H00 // DÉJEUNER

• 14H00 – 16H00 // SESSION 3 - PRIX HÉLÈNE STARCK POSTERS (SALLES VIRTUELLES)

• 16H00 – 16H20 // PAUSE

• 16H20– 17H00 // SESSION 4 - PRIX KERNER

AUDOYNAUD Charlotte
Institut Curie, Orsay
Cancérogenèse : comment se protègent les cellules ?

FARGE Thomas
Centre de Recherche en Cancérologie de Toulouse
Quand les cellules leucémiques s'évadent pour résister.

GREGORY Jules
Centre de Recherche en Epidémiologie et Statistiques, Paris
Recherche sur la chimioembolisation intra-artérielle pour le traitement du carcinome hépatocellulaire : une quantité choquante de résultats non disponibles.

LICAJ Monika
Institut Curie, Paris
Cancer de l'ovaire : on pense souvent aux cellules tumorales mais on oublie le reste ! Vers un nouveau traitement et au-delà.

LIGIER Maud
Centre de Recherche en Cancérologie de Lyon
Mélanome, le tueur qui échappe à nos défenses. Comment fait-il et comment l'arrêter ? ZEB1 une piste sérieuse

MORETTI Charlotte
Institut de Génomique Fonctionelle de Lyon
Comment les gènes sont-ils contrôlés dans nos cellules ?

PEGLION Florent
Institut Pasteur, Paris
Tumeurs cérébrales : les nageoires de l'espoir.

THYS An
Université de Nantes
Modification de SHARPIN : un pacte avec le diable ?

• 17H00 – 17H30 // ANNONCE DES LAURÉATS DES PRIX HÉLÈNE STARCK ET KERNER & DISCOURS DE CLÔTURE PAR

François DUPRÉ, Directeur Général de la Fondation ARC
& Éric SOLARY, Président du Conseil Scientifique de la Fondation ARC

JJC 2020

PRIX HÉLÈNE STARCK

SESSION 1

COMMUNICATIONS ORALES

CATÉGORIE THÈSE

MODÉRATEURS

Michaela FONTENAY - Lionel LARUE

CASANOVA ALEXANDRE
Responsable scientifique : REYNOIRD Nicolas
Institut pour l'Avancée des Biosciences, équipe Pathologies chroniques et Biomarqueurs (UMR1209), La Tronche

BCAR3 lysine methylation links SMYD2 signaling to breast cancer progression and metastasis

Context & Objectives
Post-translational modifications are a key biological mechanism increasing functional diversity of proteins, therefore participating in cell adaption to environmental changes. Lysine methylation signaling is a highly specific and dynamic process catalyzed by lysine methyltransferases, well known to impact chromatin regulation through histones methylation, as well as cell signaling pathways through non-histone proteins methylation. Importantly, deregulation of lysine methylation signaling is involved in human diseases, such as cancer. SMYD2 is a methyltransferase overexpressed in 30 % of invasive breast cancer patients and has been linked to regulation of cell motility and invasiveness of cancer cells. However, how SMYD2 impacts breast cancer progression is still elusive.

Results
In a conditional knockout Smyd2 mice model of breast cancer we showed that SMYD2 critically regulates tumor progression and metastasis spreading, suggesting important downstream signaling of SMYD2. Through proteomics approach, we found a new substrate of SMYD2 known to be crucial for breast cancer cell migration and invasion – and we confirmed this event both in vitro and in breast cancer cells. Using engineered breast cancer cell lines, we demonstrated that this new SMYD2 signalling drastically impacts migration and invasion of breast cancer cells, and identified a specific phenotype in lamellipodia, a critical cytoskeleton structure controlling cell protrusion and migration. Since methylated lysines act as a docking site for other proteins we performed a SILAC-Peptide Pulldown and identified through proteomics approach a family of specific methyl-sensitive binders of this new SMYD2 substrate. Interestingly, these proteins are known to polymerize and remodel actin cytoskeleton, and thereby tune migration ability of cells.

Conclusion & Persectives
We will now highlight the consequences of SMYD2 deregulation in the aggressiveness of breast cancer, by performing immuno-histochemistry analyses of the different actors of this new SMYD2 signaling in human breast cancer samples and patient -derived-xenograft. Furthermore, we are in the process of characterizing this pathway in mouse xenograft models and to test its relevance in metastasis formation and spreading in vivo. Better understanding of this new signaling could therefore provide important insights for future therapeutic targets in order to minimize breast cancer metastatic spreading.

Publications related to the funded project
Valentina Lukinović, Alexandre G. Casanova, *et al.* (2020). *Lysine Methyltransferases Signaling: Histones are Just the Tip of the Iceberg. Current Protein & Peptide Science.*
Roth, Gaël S, Casanova, Alexandre G et al. (2018). *Lysine methylation signaling in pancreatic cancer. Current Opinion in Oncology.*

PRIX HÉLÈNE STARCK ORAL 2020
CATÉGORIE THÈSE

CLUZET VICTORIA
Responsable scientifique: GUIGNON Céline
Université Paris Diderot, Unité de Biologie Fonctionelle et Adaptative, Equipe Physiologie de l'Axe gonadotrope, Paris

Granulosa cell tumors of the ovary: is SIRT1 a new therapeutic target ?

Context & Objectives
During her lifetime, one out of 100 women will be diagnosed with ovarian cancer. Granulosa cells tumors (GCTs) represent 7% of ovarian tumors and can affect women of all ages. This disease is far from being harmless since nearly 20% of patients die from it. This morbidity results from the high frequency of relapses and limited effectiveness of treatments. In the absence of clear data, we sought to study the molecular and cellular alterations associated with this pathology, with the aim of ultimately defining appropriate therapeutic tools. Recently, a histone deacetylase participating in the regulation of survival and proliferation processes, sirtuin 1 (SIRT1), has emerged as an interesting therapeutic target in several cancers. Our work carried out on patients' tumor tissues shows that SIRT1 is strongly expressed in human GCTs. In addition, its expression increases significantly during tumorigenesis in our transgenic mouse model of the disease. These results suggest that the SIRT1 signaling pathway could be overactivated in GCTs. Thus, our objective is now to understand the role of SIRT1 in GCTs, our hypothesis being that its inhibition would be an interesting therapeutic strategy.

Results
To answer this question, we tested in vitro and in vivo the anti-tumor efficacy of a highly specific pharmacological inhibitor of SIRT1, the Selisistat (EX-527), a molecule already successfully used in phase II clinical trials in other diseases. Our in vitro studies on granulosa tumor cell lines suggest that the use of EX-527 inhibits tumor growth by inducing cell apoptosis. Our in vivo results conducted in two mouse models of this cancer, show that inhibition of SIRT1 leads to tumor involution after four weeks of treatment, without affecting the general condition of the animals.

Conclusion & Perspectives
All of these results already show the importance of SIRT1 in promoting GCT growth progression and the anti-tumor efficacy of its pharmacological inhibition. The rest of the project aims to decipher, in vitro and in vivo, the impact of SIRT1 inhibition on the proliferation, and migration and invasion capacity of these tumor cells but also on the expression and activity of SIRT1 known targets (FOXL2, FOXO3a, E2F1 and Ku70). Overall, this project should contribute to a better understanding of the mechanisms responsible for this disease and ultimately leads to improved patient care and survival.

Publications related to the funded project
V Cluzet, MM Devillers, et al. (2020) *Aberrant granulosa cell-fate related to inactivation of p53/Rb signaling contributes to granulosa cell tumors and to FOXL2 downregulation in the mouse ovary. Oncogene.*

FARGE THOMAS
Responsable scientifique : CABON Florence
Centre de Recherche en Cancérologie de Toulouse, INSERM U1037

CD36 promotes extramedullary dissemination and relapse in Acute Myeloid Leukemia

Context & Objectives

Patients with acute myeloid leukemia (AML) are at high risk of death due to frequent relapse after chemotherapy. Most studies on chemoresistant leukemic cells are bone marrow-centered while AML is a circulating disease. We thus hypothesized that extramedullary localizations might help AML blasts to bypass chemotherapy. We previously found that chemoresistant AML blasts overexpress CD36, a fatty acid transporter and thrombospondin-1 (TSP1) receptor. CD36 being involved in metastasis and chemoresistance in other cancers, we studied its role in AML.

Results

Combining the analysis of blasts from over 360 patients, single cell RNAseq, mouse xenograft models of AML cell lines (CLDX) and patient blasts (PDX) treated with chemotherapy, we established that CD36 is associated with a poor prognosis, an increased risk of relapse, and is expressed in clones which are resistant to chemotherapy and amplified after treatment. To decipher its function, we knocked-down CD36 in three CLDX models. Inhibition of CD36 expression significantly increased survival of mice treated with chemotherapy. Surprisingly, CD36 knock-down did not affect the tumor burden in hematopoietic tissues, but significantly decreased it in several other tissues, notably in adipose tissues (AT). In cell migration assays, we found that CD36 fosters blasts' migration, TSP1 binding to CD36 being the trigger. Using in vivo antibodies blocking CD36 or TSP1 functions, we now aim at confirming in CLDX that TSP1 binding to CD36 fosters blasts dissemination upon chemotherapy, and at establishing if the inhibition of this process is a valuable therapeutic strategy. Having previously shown that chemoresistant blasts are dependent on fatty acid oxidation (FAO), we focus on AT where adipocytes might provide lipids through CD36, fueling blasts' metabolism to overcome chemotherapy and promote relapse.

Conclusion & Perspectives

Combining these complementary approaches, our aim is to unravel the importance of the extramedullary disease in AML progression and relapse after chemotherapy, to understand the contribution of CD36 to this phenotype, in particular in the adipocyte-blast dialogue, so that to propose CD36 as a new marker of prognosis and a new therapeutic target for relapsing patients.

Publications related to the funded project

Farge, T. et al. (2017). *Chemotherapy Resistant Human Acute Myeloid Leukemia Cells are Not Enriched for Leukemic Stem Cells but Require Oxidative Metabolism. Cancer Discov.*
Pascual, G. et al. (2016). *Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature.*
Ye, H. et al. (2016). *Leukemic Stem Cells Evade Chemotherapy by Metabolic Adaptation to an Adipose Tissue Niche. Cell Stem Cell.*

PRIX HÉLÈNE STARCK ORAL 2020
CATÉGORIE THÈSE

GHOROGHI SHIMA
Responsable scientifique : GOETZ Jacky
Université de Strasbourg, Inserm UMR-S1109, Strasbourg

Étude du role des GTPases Ral dans la sécrétion d'exosomes et la formation de metastases

Context & Objectives

Cancer extracellular vesicles (EVs) mainly exert pro-tumoral functions by changing the phenotypes of stromal cells to the benefit of tumor growth and metastasis. They shuttle to distant organs and fertilize pre-metastatic niches facilitating subsequent seeding by circulating tumor cells. The levels of tumor secreted EVs correlate with tumor aggressiveness, however, the link between EV secretion mechanisms and their capacity to form pre-metastatic niches remains obscure. Here, we show that GTPases of the Ral family control, through the phospholipase D1, multi-vesicular bodies homeostasis and thereby tune the biogenesis and secretion of pro-metastatic EVs. RalA and RalB promote lung metastasis in a syngeneic mouse model. Importantly, EVs from RalA or RalB depleted cells have limited organotropic capacities in vivo and, as a consequence, are less efficient in promoting lung metastasis. RalA or RalB modulate the EV levels of the adhesion molecule MCAM/CD146, which mediates lung colonization. Finally, RalA and RalB, but also MCAM/CD146, are factors of poor prognosis in human breast cancer patients. Altogether, our study identifies Ral GTPases as central molecules linking the mechanisms of EVs secretion, cargo loading to their capacity to disseminate and induce pre-metastatic niches.

Publications related to the funded project

Ghoroghi S, Mary B, et al. (2020). *Ral GTPases Promote Metastasis by Controlling Biogenesis and Organ Colonization of Exosomes. Submitted to eLife (under revision.*

HERVÉ SOLÈNE
Responsable scientifique : FACHINETTI Daniele
Institut Curie, équipe Mécanismes moléculaires de la dynamique des chromosomes, UMR 144, Paris

CENP-A chromatin prevents replication stress at centromeres to avoid structural aneuploidy

Context & objectives
Faithful chromosome segregation relies on centromeres that link chromosomes to the mitotic spindle. Human centromeres are composed of repetitive alpha-satellite DNA wrapped around the histone H3 variant CENP-A. Centromeric recombination events occur in somatic cells and are enhanced in cancerous cells where whole-arm chromosome translocations are prevalent. Changes in centromere size may elicit genome instability by increasing the rate of chromosome mis-segregation. Moreover, the mechanisms that prevent breakage and translocations at these repetitive sequences remain elusive. During my PhD, I studied the role CENP-A chromatin can play in the maintenance of centromeric DNA and in the onset of genome instability.

Results
We have combined genome editing with an auxin-inducible degron (AID) to achieve controllable, rapid and complete depletion of endogenous CENP-A in human cells. By using this system, we found that CENP-A depletion in G1 or S-phase, but not later in G2, leads to a significant increase in centromeric recombination in the following mitosis. Experiments of DNA combing revealed that the replication fork is slowed down in late S-phase at centromeres after CENP-A depletion and is concomitant with an increase in DNA-RNA hybrids, suggesting the presence of transcription-replication conflicts. Accordingly, levels of centromeric transcripts were increased and DNA damage accumulated at centromeres. Centromeric breakage during DNA replication could be rescued by overexpressing RNase H1 to remove the hybrids. Aberrant centromeric recombination was also reduced by RNase H1.
Impaired centromeric DNA replication in the absence of CENP-A led to the presence of anaphase bridges in the following mitosis and DNA damage in the ensuing cell cycle. Finally, chromosomal translocations arose specifically at centromeres after 48h of CENP-A depletion.

Conclusions & perspectives
Here we demonstrated that CENP-A is critical for the maintenance of centromeric DNA repeats by repressing R-loop formation during DNA replication, which is in turn necessary to maintain a stable karyotype. This new role for CENP-A might be relevant in cancerous cells where CENP-A is often mis-regulated and centromeres are translocation hotspots.

Publications related to the funded project
Simona Giunta, Solène Hervé et al. (2020). *CENP-A chromatin prevents replication stress at centromeres to avoid structural aneuploidy. bioRxiv*

PRIX HÉLÈNE STARCK ORAL 2020
CATÉGORIE THÈSE

JACOBS KATHRYN
Responsable scientifique : GAVARD Julie
Centre de Recherche en Cancérologie et Immunologie de Nantes Agers, UMR1232

Paracaspase MALT1 regulates glioma cell survival by controlling endo-lysosome homeostasis

Glioblastoma is one of the most lethal forms of adult cancer with a median survival of around 15 months. A potential treatment strategy involves targeting glioblastoma stem-like cells (GSC), which constitute a cell autonomous reservoir of aberrant cells able to initiate, maintain, and repopulate the tumor mass. Here, we report that the expression of the paracaspase mucosa-associated lymphoid tissue l (MALT 1), a protease previously linked to antigen receptor-mediated NF -B activation and B-cell lymphoma survival, inversely correlates with patient probability of survival. The knockdown of MALT 1 largely impaired the expansion of patient-derived stem-like cells in vitro, and this could be recapitulated with pharmacological inhibitors, in vitro and in vivo. Blocking MALT 1 protease activity increases the endo-lysosome abundance, impairs autophagic flux, and culminates in lysosomal-mediated cell death, concomitantly with mTOR inactivation and dispersion from endo-lysosomes. These findings place MALT 1 as a new druggable target involved in glioblastoma and unveil ways to modulate the homeostasis of endo-lysosomes.

Publications related to the funded project
Jacobs, KA. et al. (2020) *"Paracaspase MALT1 regulates glioma cell survival by controlling endo-lysosome homeostasis" The EMBO Journal. Journal Cover.*
Jacobs, KA, Maghe, C, and Gavard, J. (2020) *"Lysosomes in GBM: Pump Up the Volume" Cell Cycle (In press, accepted).*
Maghe, C, Jacobs, KA., Bidère, N, and Gavard, J. (2020) *"Glioblastome multiforme : Les Fleurs du MALT1" Med Sci.*

JULIEN MANON
Responsable scientifique : ZINN Sophie
Institut de Biologie Intégrative de la Cellule, CEA Saclay, Gif-sur-Yvette

Deciphering new molecular functions of the BReast Cancer protein 2 (BRCA2): role of phosphorylations during mitosis

Context & Objectives

Breast cancer is the cancer with the highest incidence and mortality rate for women around the world. Efficient strategies for the detection and treatment of sporadic cancers are already set up in industrial countries. However, hereditary cancers are more difficult to take in charge because they require a personal medical care adapted to the identified hereditary mutation(s). For providing information to the clinicians, several databases have listed the genetic variations observed in the BReast CAncer susceptibility gene 1 and 2 (BRCA1 and BRCA2). Molecular studies are then characterizing the BRCA1 and BRCA2 proteins in order to design molecular tests for variant classification.

During my PhD, I am interested in the molecular characterization of the N-terminal region of the BRCA2 protein. This region (about 1,000 residues) is predicted to be disordered, i.e. no stable 3D structure, and contains well-conserved fragments from mammals to fishes. From them the fragment BRCA2(167-260) has been shown to be phosphorylated by the kinase Plk1 at the entry into mitosis. However, phosphoresidues and their associated molecular function were not yet elucidated.

Results

Here, I used an innovative method based on Nuclear Magnetic Resonance to characterize in vitro this region. I identified that Plk1 phosphorylates BRCA2 at 4 conserved positions. This was the base for the functional characterization of these phosphoresidues.

First, I identified by biophysical methods that pT207 creates a Plk1 docking site on BRCA2. In collaboration with the group of Dr. Aura Carreira, we showed that this interaction is part of a quaternary complex involving BRCA2, Plk1, BubR1 and PP2A. In cells, this large complex regulates the Spindle Assembly Checkpoint. We also demonstrated that breast cancer variants impact the phosphorylation of BRCA2 and the formation of the complex.

Second, I initiated proteomics experiments to identify new BRCA2 partners specific of the phosphoregion. From the partners identified, I started few months ago the structural characterization of the interaction between the kinesin Kif2C and BRCA2. From my first results, I can hypothesize that a Kif2C/Plk1/BRCA2 ternary complex is formed.

Conclusion & Persectives

Finally, in this project, I fully characterized one role of the BRCA2(167-260) region. Another one is in progress. This information constitutes the base for further breast cancer variant characterization.

Publications related to the funded project

Julien et al. (2020) *1H, 13C and 15N backbone resonance assignment of the human BRCA2 N-terminal region. Biomol NMR Assign.*

Julien* et al. (2020) *Sensitivity-enhanced 13C-NMR for monitoring multisite phosphorylation. Angew Chem Int Ed Engl.*

Julien et al. (2020) *Multiple site-specific phosphorylation of IDPs monitored by NMR. Methods in Mol Biol.*

Ehlen, Julien* et al. (2020) *Proper chromosome alignment depends on BRCA2 phosphorylation by Plk1. Nat comm.*co-author*

PRIX HÉLÈNE STARCK ORAL 2020
CATÉGORIE THÈSE

LIGIER MAUD
Responsable scientifique : CARAMEL Julie
Centre de Recherche en Cancérologie de Lyon, UMR5286

Role of ZEB1-mediated cancer cell plasticity in immune escape and resistance to treatments of melanomas

Context & objectives

Melanoma is the most aggressive type of skin cancer whose treatment at a metastatic stage with targeted and immunotherapies is still faced with severe problems of resistance. Poorly described, mechanisms of resistance to immunotherapies not only rely on the composition and function of the immune infiltrate, but also on oncogenic alterations of tumor cells. By fostering adaptation, cancer cell plasticity appears as a key mechanism of resistance to treatment. Our team previously described the major oncogenic role of the transcription factor ZEB1 in melanoma. By orchestrating the cellular plasticity of melanoma cells and the reversible transition towards an undifferenciated phenotype, we also demonstrated that ZEB1 fosters resistance to targeted therapies. In line with datas in carcinoma demonstrating the role of cellular plasticity in immune escape, we hypothesized that ZEB1 mediated cancer cell plasticity could act in immune evasion of melanoma and foster resistance to immunotherapies. The objectives of my work aim to analyze if and how the expression of ZEB1 by melanoma cells: (i) modifies the composition and function of the immune microenvironment; (ii) initiates immune escape mechanisms; (iii) controls the response to immunotherapy.

Results

Analyses of the immune infiltrate in a cohort of human melanoma samples demonstrate that high ZEB1 expression in tumor cells is associated to a decreased infiltration by T CD8 lymphocytes (n=38, p=0.0162). ZEB1 gain or loss of function approaches were then implemented in vivo in syngeneic melanoma mouse models and showed that ZEB1 regulates tumor growth and modifies the composition of the immune infiltrate, in part by controlling T CD8 lymphocytes recruitment in the tumor. This may be explained at the molecular level by a decreased production of T CD8 attracting chemokines in the secretome of ZEB1-high tumors. Finally, this remodeling of the immune microenvironment has consequences on the response to anti-PD1. ZEB1 targeting was shown to synergize with anti-PD1 in inducing regression of mouse tumors.

Conclusion & Perspectives

Overall, our work demonstrates the major role of ZEB1-mediated cancer cell plasticity in melanoma immune microenvironment remodeling and opens new avenues in terms of combination strategies with immunotherapy.

JJC 2020

PRIX HÉLÈNE STARCK

SESSION 2

COMMUNICATIONS ORALES

CATÉGORIE POST-DOCTORAT

MODÉRATEURS

Marie-Caroline DIEU-NOSJEAN - Éric SOLARY

BRUNEL BENJAMIN
Responsable scientifique : PIOT Olivier
Université de Reims Champagne-Ardenne

Raman imaging for clinical histopathology: predicting colon cancer therapies response using spectroscopic markers

Context & objectives
It's difficult to know beforehand which therapy will be the most effective against cancer, and trying several ones means losing precious time in the fight against cancer. Indeed, the response to the treatment may vary widely depending on the differentiation of cancer cells, the surrounding stroma, the abundance of immune cells, or the mutational profile of cancer cells which can lead to resistances. Such cellular and stromal features of the tumor can be indirectly, but simultaneously, detected on a histological section with Raman spectroscopy which gives a molecular signature of the sample. However, providing information of clinical interest requires developing advanced processing of the spectral data. Our aim is to develop a deep learning algorithm helping the clinicians to choose the right therapy, based on the Raman spectral image of the tumor.

Results
As a first proof of concept, we focused on the more specific case of metastatic colon cancer treated with a given chemotherapy (Folfiri) associated with an anti-angiogenic agent (bevacizumab). The response to the treatment was evaluated in terms of progression-free survival (PFS), i.e. the time during which the treatment is considered as effective as the tumor does not progress. We acquired Raman spectral images of histological sections from patients presenting various responses (retrospective study). In order to improve the quality of data, we devised a genetic algorithm to optimize the pre-processing of spectra. We then trained a convolutive neural network (CNN) to predict the PFS, identifying both spatial (tissue structure) and spectral (biochemistry) features in the Raman images. The first results indicated that CNN was able to predict the right trend of PFS, but still lack precision due to the limited number of samples until now.

Conclusion & perspectives
Additional samples are required to give clinically relevant conclusions. Its acquisition and the improvement of the deep learning algorithm are underway.

PRIX HÉLÈNE STARCK ORAL 2020
CATÉGORIE POST-DOCTORAT

GORVEL LAURENT
Responsable scientifique : OLIVE Daniel
Centre de Recherche en Cancérologie de Marseille

Déterminer l'importance de l'axe TIGIT-PVR dans le développement d'Immunothérapies dans les cancers du col de l'utérus

Context & objectives
Therapeutic antibodies such as anti-CTLA-4, -Programmed cell Death (PD)-1 and –PD-Ligand1 are now used in the treatment of several tumors. However, these therapies remain inefficient in several tumors and the identification of new targets is critical. Poliovirus Receptor (PVR)-like molecules play important roles in Lymphoid cell and myeloid cell regulation. Indeed, PVR, Nectin-2 and Nectin-4, expressed on tumor cells and myeloid cell compartments, are the major ligands for TIGIT, CD96 and DNAM-1 which are expressed on the lymphoid compartment. PVR is able to bind TIGIT to induce an immunosuppressive response, but also to DNAM-1 to induce an anti-tumoral response. Nectin-2 and Nectin-4 are both able to bind TIGIT and therefore induce a pro-tumoral response. While an immunosuppressive signaling exists through PVR, none was found for Nectin-2 and Nectin-4. Note worthily, TIGIT-blockade was tested in 3 clinical trials in combination with anti-PD-L1 blockade in solid tumors and showed an improvement in patient response.

Methods
In our study, we investigate TIGIT, DNAM-1 and CD96 interactions with their ligands PVR, Nectin-2 and Nectin-4 in the context of cervical tumors. Both lymphoid and myeloid cells will be investigated, as the TIGIT-PVR axis is complex and requires a deep understanding its interactions.
Primary Tumor Infiltrating Leukocytes (TILs) were isolated using non-enzymatic digestion to preserve fragile epitopes. Phenotyping was assessed by CyTOF. Antagonistic anti-TIGIT and anti-PVR antibodies were used on primary TILs and PBMCs-Spheroid co-culture models.

Results
TIGIT could be found in CD8+ T-cells as well as Tregs, while DNAM-1 expression was almost extinguished. PVR and Nectin-2 expression was limited to dendritic cells and macrophages. Spheroid models reproduced TIGIT-PVR axis molecule expression in a similar fashion as what is found in TILs.

Conclusions & perspectives
Taken together our result show that the TIGIT-PVR axis is an interesting target in cervical tumors. Indeed, TIGIT and PVR are well expressed on TIL specific subsets and therefore allow a better understanding of which cell type will be affected if they are targeted by an antibody. As TIL only offer few cells for functional assays with antibodies, our spheroid model should provide a very interesting alternative and provide the answer to which molecules of the TIGIT-PVR axis are the best targets for immunotherapies.

KRAMARZ KAROL
Responsable scientifique : LAMBERT Sarah
Institute Curie, UMR3348, Orsay

Processing of replication forks at the nuclear periphery: Mechanism and impact on genome stability

Nuclear Pore complexes (NPCs) act as docking sites to anchor particular DNA lesions facilitating DNA repair by elusive mechanisms. Using replication fork barriers in fission yeast, we report that relocation of arrested forks to NPCs occurred after Rad51 loading and its enzymatic activity. The E3 SUMO ligase Pli1 acts at arrested forks to safeguard integrity of nascent strands and generates poly-SUMOylation which promote relocation to NPCs but impede the resumption of DNA synthesis by homologous recombination (HR). Anchorage to NPCs allows SUMO removal by the SENP SUMO protease Ulp1 and the proteasome, promoting timely resumption of DNA synthesis. Preventing Pli1-mediated SUMO chains was sufficient to bypass the need for anchorage to NPCs and the inhibitory effect of poly-SUMOylation on HR-mediated DNA synthesis. Our work establishes a novel spatial control of Recombination-Dependent Replication (RDR) at a unique sequence that is distinct from mechanisms engaged at collapsed-forks and breaks within repeated sequences.

Publications related to the funded project

Kramarz K et al. *The nuclear pore primes recombination-dependent DNA synthesis at arrested forks by promoting SUMO removal.* Nature Communications 2020 (accepted manuscript)
Ait-Saada A et al. *Chromatin remodeler Fft3 plays a dual role at blocked DNA replication forks. Life Sci Alliance.* 2019
Kramarz K et al. *The Analysis of Recombination-Dependent Processing of Blocked Replication Forks by Bidimensional Gel Electrophoresis. Methods Mol Biol.* 2021

PRIX HÉLÈNE STARCK ORAL 2020
CATÉGORIE POST-DOCTORAT

MOINDJIE HADIA
Responsable scientifique : NAHMIAS Clara
Gustave Roussy, équipe Prédicteurs moléculaires et nouvelles cibles en oncologie, U981, Villejuif

Targeting cell metabolism to overcome taxane-based chemoresistance in high-grade serous ovarian cancer

Context & objectives

High-grade serous ovarian cancer (HGSOC) is the fourth leading cause of cancer death in women. Currently, the only therapy available for these tumors is chemotherapy followed by surgery. Despite high initial response rates, patients acquire resistance to chemotherapy, relapse and eventually succumb.
Chemotherapy for ovarian cancer includes platinum salts (DNA damaging agents) and taxanes that stabilize microtubules. We hypothesized that altered expression of microtubule-regulating proteins (MT-reg) in HGSOC may affect microtubule organization and function, thereby conferring resistance to taxane-based chemotherapy.

Methods

Transcriptomic analysis of two independent cohorts of HGSOC patients was performed to compare the expression levels of 411 genes encoding MT-reg in tumours classified as sensitive or resistant to chemotherapy. Mitochondrial metabolism and activity were assessed by seahorse measurement and flow cytometry. Mitochondrial structure and dynamic were observed by confocal imaging, electron microscopy and videomicroscopy. Immunoprecipitation followed by mass spectrometry allowed us to identify 301 specific protein partners of the best candidate in HGSOC cells.

Results

Among 53 MT-reg genes dysregulated in HGSOC, we focused our interest on Syntabulin (SYBU) which is over-expressed in resistant HGSOC. SYBU is a mitochondrial protein that interact with kinesin to control mitochondrial transport along MT in neurons. However its role in cancer has never been described. We show here that SYBU localizes along the microtubule lattice and is part of protein-protein interaction networks centered around mitochondrial and MT-reg proteins. SYBU depletion impairs the MT-stabilizing effects of taxanes. SYBU-depleted cancer cells also show decreased mitochondrial oxygen consumption rate and are more sensitive to the metabolic effects of taxanes. SYBU depletion does not impact mitochondrial mass suggesting that SYBU regulates mitochondrial activity rather than quantity. Ongoing live cell imaging studies aim at exploring the effects of SYBU mitochondrial intracellular transport along microtubule.

Conclusions & Perspectives

Together these findings suggest that taxane resistance induced by SYBU in HGSOC may be due in part to increased oxidative phosphorylation and microtubule stabilization. We highlight a potential link between microtubule dynamic and mitochondrial trafficking in response to taxane-based chemotherapy.

PEGLION FORENT
Responsable scientifique : ETIENNE-MANNEVILLE Sandrine
Institut Pasteur, Polarité Cellulaire, Migration et Cancer, Paris

Role of PTEN loss in Glioblastoma Invasion

Context & Objectives
Glioblastoma (GBM) are malignant brain tumour of glial origin. They bear a dismal 15months prognosis and cause the death of 225 000 patients each year. Its late diagnosis due to lack of early symptoms, its redundant genetic anomalies, its high heterogeneity, the lack of accurate preclinical model mimicking the human brain and more specifically its highly invasive nature explain the absence of major therapeutic success. GBM cells migrate anarchically in vitro highlighting their inability to maintain the axis of polarity required for directed cell migration. We hypothesized that the alteration of cell polarity is a driver of GBM invasivity and aim to decipher its molecular origin. PTEN is a key molecule for cell polarity and migration, which is altered in more than 60% of GBM. The project aims at decoding the role of PTEN loss in the GBM invasive nature.

Results
Our data show that PTEN loss increases collective cell migration speed of healthy glial cells and alters both transverse actin cables polarisation and adherens junction dynamics. PTEN is a phosphatase that targets both lipids and proteins. Mechanistically we observed that PTEN slows down glial cell migration only via its protein phosphatase function, independently of the PI3K/AKT pathway usually at play in PTEN depleted tumours. PTEN loss enhances AMPK activity, which increases the phosphorylation of the actin regulator VASP. In PTEN-depleted GBM cells, AMPK inhibition prevents GBM invasion in vitro. I then developed two in situ tumour dissemination assays to validate the implication of AMPK in vivo. The first model consists in the xenotransplantation of primary GBM cells in the zebrafish larvae brain, coupled to high-resolution intravital imaging. This assay allows to gauge the long-term invasivity pattern of the GBM cells with or without treatment, but also simultaneously to study their mode of migration in situ at the cellular and subcellular scale in real time. I finally developed a complementary model using human brain organoid (“minibrains”) co-cultured with GBM tumour organoid.

Conclusion & Perspectives
The knowledge we will gain about the molecular mechanism controlling GBM invasion in situ should pave the way to the development of more successful therapies. Using the zebrafish assay to test multiple drugs diluted in fish water on numerous larvae, we aim to develop strategies to tackle AMPK in vivo.

PRIX HÉLÈNE STARCK ORAL 2020
CATÉGORIE POST-DOCTORAT

SMITH REBECCA
Responsable scientifique : HUET Sébastien
Institut de Génétique et Développement de Rennes

The chromatin remodeler ALC1 underlies resistance to PARP inhibitor treatment

Context & objectives
Poly(ADP-ribose) polymerase 1 (PARP1) is a key actor in the DNA damage response. PARP1 is rapidly recruited to sites of damage where it catalyses the addition of poly(ADP-ribose) (PAR) chains on target proteins within close proximity to the break site. These PAR chains signal the presence of the break site to down-stream repair factors, as well as triggering chromatin remodeling to facilitate access to DNA breaks. If PARP1 recruitment to sites of damage is important for repair initiation, its timely release is also crucial to avoid deleterious consequences. Indeed, the cytotoxicity of PARP inhibitors used as anticancer drugs is thought to originate from the trapping of inhibited PARP1 at DNA lesions, which prevents further repair. Currently, PARP inhibitors are utilized in the treatment of BRCA-deficient cancers with treatments currently extending towards other homologous recombination defective tumors. In this study, we aim to identify additional factors that could regulate the efficacy of PARP inhibitor treatment and understand the associated underlying molecular mechanisms.

Results
In a genome-wide CRISPR knockout screen with the PARP inhibitor olaparib, we identify ALC1 – a cancer-relevant poly(ADP-ribose)-regulated chromatin remodeling enzyme, as a key modulator of sensitivity to PARP inhibitor. We discovered that ALC1 can remove inactive PARP1 indirectly through binding to PARylated chromatin. Consequently, ALC1 deficiency enhances trapping of inhibited PARP1, which then impairs the binding of both non-homologous end-joining and homologous recombination repair factors to DNA lesions. We also establish that ALC1 overexpression, a common feature in multiple tumor types, can reduce the sensitivity of BRCA-deficient cells to PARP inhibitors.

Conclusions & perspectives
Taken together, we conclude that ALC1-dependent PARP1 mobilization is a key step underlying PARP inhibitor resistance. Additionally, our study argues for a systematic analysis of the ALC1 expression level prior to the use of PARP inhibitor-driven cancer therapies.

JJC 2020

PRIX HÉLÈNE STARCK

SESSION 3

COMMUNICATIONS POSTERS

SALLE VIRTUELLE 1

MODÉRATEURS

Joseph CICCOLINI - Géraldine GENTRIC - Éric SOLARY

AL JORD ADEL // Post-doctorante
Responsable scientifique : VERLHAC Marie-Hélène
Collège de France, Centre interdiciplinaire de Recherche en Biologie, Paris

Cytoplasmic remodeling orchestrates functional sub compartmentalization of the oocyte nucleus

Context & Objectives
Cycling and differentiating cells remodel their cytoplasm via cytoskeleton-associated molecular motors. The remodeling generates stochastic force fluctuations that actively stir the cytoplasm. Metastatic cancer cells are known to intensify these cytoplasmic forces that subsequently induce organelle membrane bending and transport. This project's goal was to investigate the unknown consequences of these cytoplasm-based random force fluctuations on the interior of organelles.

Results
We uncovered that mammalian female germ cells, named oocytes, intensified cytoplasmic force fluctuations to drive liquid-phase compartmentalization inside their nucleus. Using a functional single live-cell approach together with computer simulations and bioinformatics, we probed multiple biomolecular condensates responsible for nucleus sub-compartmentalization during mouse-oocyte cytoplasmic remodeling. Cytoplasmic force intensification enhanced multiscale kinetics of nuclear liquid-like condensates, boosting micron-scale fusion of condensates while stirring and concentrating their core with key functional molecules. Amplifying cytoplasm-based fluctuations accelerated nucleus compartmentalization, whereas disrupting them disorganized nuclear liquid-phase compartments and altered messenger RNA processing. Evidence suggesting evolutionary conservation of this nucleus-compartmentalizing mechanism was found in insects.

Conclusion & Perspectives
We show that cells can deploy cytoplasm-sourced stochastic forces to functionally remodel liquid-phase compartments in membrane-bound organelles such as the nucleus. Beyond the fundamental insight, our study identifies cytoplasmic remodeling as an orchestrator of nuclear liquid-phase compartmentalization in animal cells. This finding thus grants new perspectives for neurodegeneration-related studies, where cytoskeletal mechanics and subnuclear compartmentalization can be perturbed, and for tumor-related studies, since both modulation of cytoplasmic forces and disruption of nuclear compartmentalization were independently linked to cancer.

PRIX HÉLÈNE STARCK 2020

ALRIC HADRIEN // Master 2
Responsable scientifique : RAHMI Gabriel
Paris Centre de Recherche Cardiovasculaire, équipe Imagerie de l'angiogenèse, UMR 970, Paris

Thermoresponsive gel embedding adipose stem cell-derived extracellular vesicles improves the healing of colonic anastomosis following irradiation in rats

Context & Objectives
The incidence of rectal cancer in European Union is 125000 per year. Locally advanced disease is recommended to undergo neoadjuvant chemoradiotherapy supplemented by surgery with the confection of anastomosis. Anastomotic fistula incidence varies from 3 to 21% and is associated with increased mortality. Cellular therapy using mesenchymal stromal cells (MSCs) showed promising results in terms of anastomotic fistula healing. Extra-cellular vesicles (EVs) derived from MSCs and reproducing their paracrine effect therefore appears to represent an interesting alternative. A thermoresponsive gel, the Pluronic ®F127 (PF-127), embedding EVs would ensure optimal application on the anastomosis.
To evaluate the feasibility and efficacy of the application of PF-127 gel with or without EVs to improve healing of colonic anastomoses on irradiated colon in rats.

Methods
40 Sprague-Dawley rats were submitted to a 3 x 12,5 Gray irradiation centered on the colorectal area. Rats were randomized in four experimental groups of 10 rats: a control untreated group, a treated group with PF-127, a treated group with EVs alone and a treated group with PF-127 gel with EVs (Gelveco). Three weeks after irradiation, a colonic anastomosis in irradiated area was performed and the treatment was applied. Eight weeks after irradiation, we evaluated the efficacy of each treatment. The primary endpoint was the healing of the anastomosis during colonoscopy.

Results
Two rats died in each untreated and PF-127 groups whereas 1 rat died in each EVs and Gelveco groups. Eight weeks after irradiation, in the Gelveco group we observed a lower endoscopic inflammatory index (untreated: 3.8 +/-1.1, PF-127 3.9 +/-1.5, EVs 2.9 +/-1.5, Gelveco 2.4+/-1.1, p=0.07). In addition, the rats autopsy showed lower peritoneal inflammation (Zuhlke score) in the EVs and Gelveco groups (untreated: 2.2 +/-0.4, PF-127: 1.9 +/-0.4, EVs: 1.1 +/-0.2, Gelveco: 1.2 +/- 0.2, p=0.04). The size of anastomotic ulcers (µm) in histology was smaller in EVs and Gelveco groups (untreated: 1191 +/-129, PF-127: 1353 +/-165, EVs: 683 +/-128, Gelveco: 716 +/- 104, p<0.001) while the fibrotic infiltrate (%) was only smaller in the Gelveco group (untreated: 54.7 +/-2.8, PF-127: 58.2 +/-3.5, EVs: 53.6 +/-2.3, Gelveco: 46.7 +/-2.9, p=0.04).

Conclusion
The application of EVs promoted by an appropriate thermoresponsive gel appears promising for the healing of post-radiotherapy digestive anastomosis.

DUFRESNE SUZANNE // Doctorante
Responsable scientifique : REBILLARD Amélie
Université Rennes 2, Laboratoire M2S

Voluntary wheel running does not enhance radiotherapy efficiency in a PC-3 preclinical model: towards a tailored approach to physical activity?

Improved tumor vascularization has been hypothesized to enhance the radiotherapy (RT) response in prostate cancer (PCa). Several preclinical models have shown that physical activity has the ability to modulate tumor blood flow but its impact on RT efficiency remains largely unknown. In our study, athymic mice were injected with PC-3 cells and either remained inactive or were housed with voluntary running wheels. Echography of the tumor revealed that despite no observed effect on tumor growth, voluntary wheel running (VWR) enhanced tumor perfusion. We thus evaluated whether VWR could increase RT efficiency in a similar PC-3 cancer model. Contrary to our hypothesis, no effect on the RT response was observed. These findings contrast with previously published results by our team and prompted us to evaluate (1) if a different physical activity modality (treadmill running) could slow down tumor growth and (2) whether the potential anti-neoplastic effects of physical activity are dependent on PCa type. Treadmill running did not significantly slow down tumor growth in mice bearing PC-3 tumors, similar to what was observed for VWR. Interestingly however, in vitro experiments suggested that while PC-3 cells were not responsive to chronic exercise-conditioned media, LNCaP had a significant reduction in their proliferation. Hence, the potential anti-cancer effects of physical activity may be dependent on PCa type. A better understanding of which PCa cell lines are sensitive to the anti-cancer effects of physical activity in vitro and in vivo may help to develop a personalized approach to physical activity in the clinical setting.

Publications related to the funded project

Dufresne S., Guéritat J., et al. (2020) *Exercise training improves radiotherapy efficiency in a murine model of prostate cancer. FASEB J.*
Assi M., Dufresne S., Rebillard A. (2020) *Exercise shapes redox signaling in cancer. Redox Biol.*

PRIX HÉLÈNE STARCK 2020

FABRE BERTRAND // Post-doctorant
Responsable scientifique : PLAZA Serge
Laboratoire de Recherche en Sciences Végétales (LRSV), Auzeville-Tolosane

Exploring the hidden proteome: identification of microproteins using mass-spectrometry

Context & Objectives

A novel class of molecules, short open reading frame (smORF)-encoded polypeptides (SEPs, also known as microproteins), has recently emerged as a new potential key player in biology. Indeed, several recent studies have revealed the importance of these intracellular peptides in the regulation of key cellular processes, such as mTOR signaling, DNA damage repair or apoptosis, all being major pathways studied in cancer biology.

Methods

Computational methods based on prediction and conservation for the annotation of sORFs combined with transcriptomics and ribosome profiling approaches ended up in the prediction of thousands of SEPs among several species. For example, the expression of 73,582 SEPs are predicted in Drosophila melanogaster. However, we still lack unambiguous evidence for the existence of most predicted SEPs. So far, only hundreds of SEPs have been validated (e.g. 132 in Drosophila melanogaster). The roles of fewer SEPs, less than 50 across all species, have been characterized in details. SEPs still represent a largely unexplored repertoire of active biomolecules with possible important roles in the animal and plant kingdoms.

Results

One of the main goal of the host laboratory, headed by Drs. Jean-Philippe Combier and Serge Plaza (LRSV, Auzeville-Tolosane), two experts in SEPs biology, is to discover new SEPs and study their functions in plants and animals. Earlier this year, I joined their group as a proteomics expert aiming at identifying SEPs by mass spectrometry in Drosophila melanogaster and Human. In a first attempt to identify microproteins, we optimized several biochemical methods to enriched peptides from cell lysates of drosophila flies and cultured embryonic cells. Since I started in the group, and in collaboration with the proteomics facility (IPBS, Toulouse), we were able to identify more than 100 yet uncharacterized SEPs in Drosophila melanogaster. By developing more elaborated workflows, we are now working on increasing the number of newly discovered peptides in Drosophila melanogaster and transfer these approaches to human samples.

Conclusion

In parallel, we performed the interactome of one of the newly validated SEP and identified several key proteins of the mTOR signaling pathway as associated proteins. We are now assessing the function of this SEP as a potential new regulator of the mTOR pathway in Drosophila.

LEBDY RANA // Doctorante
Responsable scientifique : RIBEYRE Cyril
Institut de Génétique Humaine, équipe Instabilité Génétique et Cancer, Montpellier

GNL3: a new player in the protection of stalled replication forks

DNA replication requires a plethora of proteins to maintain its accuracy especially in presence of impediments that increase replicative stress. This is particularly important for stem cells maintenance or response to chemotherapies that target specifically cancer cells by increasing their level of replicative stress. Although chemotherapies are efficient in most cases, some cancers develop resistance. Therefore, there is a need to identify new proteins at replication forks that may influence the response to chemotherapeutic drugs. Using the iPOND (isolation of proteins on nascent DNA) technique coupled to mass spectrometry we uncovered new proteins associated with replication forks. We used a secondary screen to identify the best candidates and selected GNL3 (aka nucleostemin) for further analysis.
GNL3 is involved in the maintenance of genomic integrity in cancer cells where it is found to be overexpressed, but its precise role(s) in this process is poorly understood. We demonstrated that GNL3 is associated with ongoing replication forks using iPOND and Proximity Ligation Assay (PLA). The depletion of GNL3 does not impair replication forks progression in basal conditions or in response to short treatments with camptothecin. However, prolonged treatment with hydroxyurea (HU), etoposide or camptothecin elevates the phosphorylation of RPA suggesting an increase in the frequency of collapsed forks. In agreement with this, we found that prolonged HU treatment resulted in a nucleases dependent DNA resection in absence of GNL3. Interestingly we demonstrated that GNL3 impairment increases the number of replication forks suggesting a role in regulation of origin firing possibly via an interaction with ORC2. We propose a model that explains how GNL3 prevents replicative stress by regulating replication origins.

PRIX HÉLÈNE STARCK 2020

PETRAZZUOLO ADRIANA // Doctorante
Responsable scientifique : KROEMER Guido
Centre de Recherche des Cordeliers, U1138, Paris

Mechanisms of immunogenicity of ALK-positive Anaplastic large cell lymphoma

Context & Objectives

Anaplastic large cell lymphoma (ALCL) is a pediatric tumor driven by the expression of Anaplastic Lymphoma Kinase (ALK). Currently, chemotherapy is the standard of care, but it causes deleterious side effects. This is the reason why targeted therapies are being tested as therapeutic options. ALK inhibitors have been shown to specifically kill cancer cells, inducing a sizable response, but patients relapse due to resistance. Immune response boosters, such as immune-checkpoint blockers, have also been proven to be beneficial, producing a durable response. Nevertheless, they are effective only in a small fraction of patients. The overall objective of my thesis work is to investigate the immunogenicity of ALK inhibitors in order to build scientific basis to combine kinase inhibitors and immunotherapies, finally achieving complete remission in most ALCL patients. Indeed, we have already demonstrated that ALK inhibitors in vitro induce immunogenic cell death, process able to stimulate a specific anti-tumor immune response. Moreover, we have found that ALK inhibitors induce regression of ALK+ ALCL tumors in vivo in mouse models. However, invariably, tumors grew back due to resistance, mirroring patients' scenario. Accordingly, we propose to explore the combination of ALK inhibitors and immune checkpoint blockers as potential synergistic approach for the treatment of ALK+ ALCL tumors.

Conclusion

Our data demonstrated that either via their physicochemical features or by specific recognition of membrane receptors, CPB and GBVA10-9, are capable of increasing the uptake of nanovectors and bioconjugates by hepatoma cells and could therefore become suitable targeting agents for diagnosis and/or treatment of HCC.

TRAN VU LONG // Post-doctorante
Responsable scientifique : TRUILLET Charles
Service Hospitalier Frédéric Joliot, CEA/INSERM, Laboratoire Biomaps, Orsay

Development of novel solutions for improving the diagnosis and treatment of brain cancers

Context & Objectives
Brain cancers are the leading cause of deaths due to their aggressiveness and the vital functions of brain. Different molecular targets have been discovered for brain cancer diagnosis and therapy. However, the prognosis of these types of cancer are still gloomy. Several reasons could be pointed out for this slow progress i.e. the variability in cellular origin of the tumors, low penetrability of therapeutic molecules across the blood brain barrier (BBB), the lack of specificity of certain treatments such as external beam radiotherapy as well as the lack of translatable imaging strategies for evaluating the biodistribution of therapeutics.

Methods
The postdoctoral project is to bring new perspectives in the care of patients with cerebral tumor including:
- Valuable diagnosis able to distinguish the different brain tumor, especially between the cerebral lymphoma and the glioma. We are studying the potential of [18F]Fludarabine for distinguishing brain lymphoma from glioma in preclinical model. This might be essential for choosing the most adapted treatment in clinical scenario.
- Innovative theranostic approach to treat cerebral tumor 1) with nanoparticles for external irradiation therapy or 2) with immunotherapy. We have developed new and highly applicable radiolabeling methods using bio-orthogonal 18F or 89Zr chemistry for imaging therapeutic antibodies and nanoparticles (NPs). Studied NPs were proven to improve the specificity and reduce the side effects of radiotherapy. Our imaging strategy allow visualizing and quantifying spatially and temporally the tumor uptake of these NPs, which is crucial for optimizing the use of such NPs. For immunotherapy, the main limitation is the poor penetrability of monoclonal antibody across BBB. We use focused ultrasound (FUS) to induce locally reversible disruption of BBB, which allows safely enhancing brain delivery of cetuximab. For demonstrating the efficiency of this technique, we labeled cetuximab with 89Zr and visualize the product by in vivo PET.

Publications related to the funded project
Tran et al. (2020). *Impact of blood-brain barrier permeabilization induced by ultrasound associated to microbubbles on the brain delivery and kinetics of cetuximab: An immunoPET study using 89Zr-cetuximab. Theranostics.*
Tran et al. (2019). *A snake toxin as a theranostic agent for the type 2 vasopressin receptor Chemical Communications. New fluorine-18 pretargeting PET imaging by bioorthogonal chlorosydnone–cycloalkyne click reaction*

VENE ÉLISE // Doctorante
Responsable scientifique : LOYER Pascal
Institut NuMeCan, UMR1241, Rennes

In vitro evaluation of synthetic peptides as active-binding agents in bioconjugates and polymeric nanoparticles to target hepatoma cells

Context & Objectives
Hepatocellular carcinoma (HCC) is the main primary malignant tumor of the liver. Despite major progress in diagnosis and treatment, effective HCC therapies remain limited in part because of the chemoresistance and the poor distribution of drugs at the tumor site. Active cell targeting strategies are interesting to deliver high drug concentrations to specific cells while protecting patients from side effects.

Methods
We selected 12 synthetic peptides from the literature for their putative tropism towards hepatocytes in order to study their ability to target hepatic cancer cells in vitro. A screening was done by conjugating the peptides with fluorescent streptavidin and evaluating the cellular uptake of these complexes by flow cytometry.

Results
Our results showed that two peptides, CPB and GBVA10-9, had a clear "hepatotropism". As peptides are interesting targeting agents to functionalize NPs, we engrafted all peptides onto surface of polymeric NPs using biotin-streptavidin-based bridging system. Evaluation of NPs uptake confirmed our previous results: CPB and GBVA10-9 enhanced significantly the cellular uptake of NPs. Then, we generated negative-control peptides of CPB and GBVA10-9 by redistribution or substitution of their amino acids (AA) sequences. Studies of peptide-functionalized NPs cell uptake demonstrated that GBVA10-9 and its negative-control strongly bound to liver cancer cells and suggested that their physicochemical features are the main drivers of the NPs cell internalization rather than a membrane receptor recognition mediated by a specific peptide sequence, unlike CPB for which the AA sequence is determinant for the cell uptake. Considering these surprising results, we assessed more specifically the peptides' tropism towards hepatic cells in absence of NPs. In vitro experiments were performed using fluorescent streptavidin peptide conjugates in order to determine whether the cell internalizations of CPB and GBVA10-9 were significantly different from those of their control counterparts. In contrast with results obtained with NPs, cellular uptake of these conjugate complexes was higher with CPB and GBVA10-9 than with their negative-control.

Conclusion
Our data demonstrated that either via their physicochemical features or by specific recognition of membrane receptors, CPB and GBVA10-9, are capable of increasing the uptake of nanovectors and bioconjugates by hepatoma cells and could therefore become suitable targeting agents for diagnosis and/or treatment of HCC.

JJC 2020

PRIX HÉLÈNE STARCK

SESSION 3

COMMUNICATIONS POSTERS

SALLE VIRTUELLE 2

MODÉRATEURS

Paola ARIMONDO - Lionel LARUE - Robert FEIL

DUMETIER BAPTISTE // Doctorant
Responsable scientifique : DUBREZ Laurence
Université de Bourgogne, équipe Lipides, nutrition, cancer, U1231, Dijon

Role of TRAF2 in cIAP1 oncogenic properties

Context & Objectives

cIAP1 (« cellular inhibitor of apoptosis 1») is an E3-ubiquitin ligase with oncogenic properties. It is involved in proliferation, differentiation, cell death, migration and inflammation. In humans its expression has been correlated with invasiveness and resistance to chemotherapy.
TRAF2 allows the recruitment of cIAP1 in TNFR-associated protein complexes. cIAP1-TRAF2 interaction has been observed in several cellular models independently of receptor activation. The aim of this project is to study the role of TRAF2 in cIAP1 oncogenic activity.

Methods

Mouse embryonic fibroblast (MEF) depleted in cIAP1 or in cIAP1 and cIAP2 were transformed by the HRas-V12 oncogene and infected with several IAP mutants encoding constructs. We checked their ability to generate tumors in mice xenograft and metastasis models. The activation of signaling pathways was analyzed. TRAF2 proteome was investigated by immunoprecipitation and by mass spectrometry.

Results

cIAP1 expression in MEF cIAP1-/- or cIAP1-/-/cIAP2-/- increased tumor development in nude mice xenograft model, and promoted lung node formation when IV injected. cIAP1 interacts with TRAF2 via its BIR1 domain. BIR1 deletion or mutation within the TRAF2 binding site completely abolished cIAP1 oncogenic properties. A comparative analysis of MEF-cIAP1 cells versus MEF-cIAP1-mutants showed that cIAP1 fosters the activation of NF-kB, ERK and JAK/STAT3 pathways without modulating p38MAPK or SAPK/JNK. cIAP1 expression induced the formation of TRAF2 clusters in the cytoplasm suggesting its recruitment in signaling platforms. We analyzed TRAF2 interactome. Its partners are involved in various signaling pathways and participate to different cellular processes. cIAP1 deletion decreased by 75% the number of TRAF2 partners within all signaling pathways. Structural analysis of cIAP1-TRAF2 interaction shows that BIR1 can bind 3 TRAF2 molecules. TRAF2 aggregation was induced by expressing an isolated BIR1 domain. The expression of the BIR1 domain stimulated tumor growth suggesting that TRAF2 clustering is sufficient to initiate a pro-tumoral signaling pathways.

Conclusion

These results show that TRAF2 is necessary for cIAP1 oncogenic activity. Conversely, cIAP1 allows TRAF2 aggregation and activation. This clustering is sufficient to stimulate tumor progression. These results assume that inhibiting cIAP1-TRAF2 could slow down tumor growth, and bring new perspectives into the research of cancer-targeted therapies.

Publications related to the funded project

B Dumétier, A Zadoroznyj, et al. (2020). *The role of cIAP1 – TRAF2 interplay in tumor growth.* (submitted).

GIACCHERIN CÉDRIC // Doctorant
Responsable scientifique : GAILLARD Pierre-Henri
Centre de Recherche en Cancérologie de Marseille

Control of Mus81-Eme1 complex to ensure genome stability

Context & Objectives

A potentially important source of genome instability lies in the formation of secondary DNA structures during DNA metabolism. These structures, which are most of the time normal intermediates, need to be processed to ensure the restoration of an intact DNA molecule. The resolution of secondary DNA structures is achieved by so-called structure specific endonucleases. However, although pivotal for genome stability, these enzymes may be viewed as double-edged swords, which unless properly controlled may fuel genomic instability. While an increasing number of studies shed light on endonucleases misregulation in cancer etiology, much remains to be done to understand how these enzymes are controlled. We are tackling these fundamental questions by using the yeast Schizosaccharomyces pombe.
Mus81-Eme1 is a heterodimeric endonuclease that can process branched DNA structures such as Holliday Junction (HJ). In fission yeast, Mus81-Eme1 activity is essential for cell viability in absence of the Rqh1 helicase. This is the orthologue of the human BLM helicase that is defective in Bloom syndrome patients, a heritable syndrome associated with a remarkable chromosomal instability and cancer predisposition.

Methods

We uncovered a control mechanism of Mus81-Eme1 based on an initial cell cycle driven phosphorylation of Eme1 by Cdc2(CDK1) that primes Eme1 for further phosphorylation by the Rad3(ATR)-Chk1(CHK1) pathway upon DNA damage. This dual phosphorylation is crucial in absence of Rqh1 to prevent gross chromosomal rearrangements (GCR). In order to gain further insight into the control of Mus81-Eme1, we purified recombinant Mus81-Eme1 from bacteria and isolated active Cdc2, Rad3 and Chk1 kinases from yeast. In an attempt to reconstitute in vitro the dual phosphorylation of Eme1, we confirmed that Eme1 is a substrate of Cdc2 and realized that Eme1 is also a direct target of Rad3, in a Chk1-independent manner.

Results

We identified in silico eight SQ/TQ Rad3 consensus in Eme1. These sites are all phosphorylated by Rad3 in vitro and mutating these sites strongly reduces Eme1 phosphorylation in vivo in response to DNA damage without affecting the Cdc2-dependent phosphorylation of Eme1. Strikingly, Eme1 phosphorylation is crucial to prevent GCR in absence of Rqh1. These results unveil a new layer of Mus81-Eme1 control, based on Rad3 direct phosphorylation of Eme1. We are currently investigating the functional relevance of the phosphorylation of Eme1 on the activity of Mus81-Eme1 in vitro.

GUILLORY XAVIER // Post-doctorant
Responsable scientifique : CHEVET Eric
Université de Rennes 1, Unité Inserm U1242 & UMR CNRS 6226

Novel IRE1 pharmacological inhibitors for adjuvant therapy in glioblastoma

Context & Objectives
Glioblastoma multiform (GBM) represents the most frequent and malignant form of primary brain tumors with a median survival of 15 to 18 months, despite aggressive treatments. Inositol-requiring enzyme 1 (IRE1) is a bifunctional serine/threonine kinase and endoribonuclease that is a major mediator of the unfolded protein response during endoplasmic reticulum (ER) stress. Tumour cells experience ER stress due to adverse environmental cues such as hypoxia or nutrient shortage and high metabolic/protein-folding demand. To cope with those stresses, cancer cells utilise IRE1 signalling as an adaptive mechanism and IRE1 has been proven to play an instrumental role in several cancers, including GBM.

Methods
We recently demonstrated through intracerebral inhibition that IRE1 is a highly relevant therapeutic target for adjuvant treatment in GBM as it slows down tumor growth and sensitize tumor cells to the current treatment.
Unfortunately, known modulators of IRE1 activity cannot cross the blood-brain barrier (BBB) and are therefore incompatible with concomitant administration as adjuvant. In this context, we developed a structure driven drug discovery pipeline to identify novel inhibitors able to cross the BBB. This study yielded a new class of non-toxic compounds, Z4, showing inhibitory activities in GBM cell models, sensitization of tumor cells to chemotherapy, and promising results in mice models. Although highly promising entry points, several key aspects need to be addressed. In particular, the structure-activity relationships (SAR); potency; kinase selectivity; and PK/PD profiles.
Starting with the SAR study, whose purpose is to identify the molecular contribution (H-bonds, hydrophobic, etc.) of each component to the binding, four parts with straightforward synthetic access have been identified in Z4 molecular scaffold, allowing diversified pharmacomodulation. The choice of analogues has been made in synergy with molecular modelling and syntheses are ongoing. This approach is complemented by directed mutagenesis to alter key residues in IRE1's binding pocket. Finally, NMR experiments with wild type and mutant IRE1 proteins are about to start and should yield useful knowledge about the binding of these molecules in solution.

Results
These early steps will give us a deeper understanding on how to target IRE1 and develop improved inhibitors for testing in our preclinical animal models. Lead candidates could directly enter early clinical trial on site.

Publication related to the funded project
Raymundo, D. P.; Doultsinos, D.; Guillory, X.; et al. (2020). *Pharmacological Targeting of IRE1 in Cancer. Trends in Cancer.*
Raymundo, D. P.; Eriksson, L. A.; Chevet, E.; Guillory, X (2020). *Structure-based drug discovery of IRE1 modulators. Methods in Molecular Biology.* (Submitted).

PRIX HÉLÈNE STARCK 2020

LICAJ MONIKA // Doctorante
Responsable scientifique : MECHTA-GRIGORIOU Fatima
Institut Curie, équipe Stress et Cancer, U830, Paris

Impact of chemotherapy on fibroblast heterogeneity and immune infiltration in high-grade serous ovarian cancer

Context & Objectives
High grade serous ovarian cancer (HGSOC) is the leading cause of death from gynecological cancer. Ovarian adenocarcinomas are complex tissues, composed not only of tumor cells but also of a stromal and immune microenvironment. It has been identified in our lab, four main subpopulations of fibroblasts or CAF (Cancer Associated Fibroblasts), referred to as CAF-S1 to -S4. Although CAF-S2 and CAF-S3 can be also detected in healthy tissues, CAF-S1 and CAF-S4 are strictly found in tumors.

Methods
The first objective of my work was to analyze the impact of chemotherapy on the stromal and lymphocytic microenvironment in HGSOC. The study of the retrospective and prospective cohort allowed us to demonstrate that after treatment, the proportion of myofibroblastic CAF subpopulations (CAF-S1 and CAF-S4) significantly decreases and immune cells re-infiltrate the residual tumor tissue. In addition, we showed that the decrease in CAF-S1 after treatment is correlated with the increase in lymphocyte infiltration, particularly cytotoxic CD8+ T cells. Interestingly, transcriptomic analyzes of the stroma of an ovarian cancer PDX recapitulating the tumor characteristics of the original patient, confirm that the treatment reduces the proportion of CAF-S1 which has immunosuppressive properties. CAF-S1 are characterized by the activation of the YAP / TAZ TEAD signaling pathway. Moreover, the expression of YAP1 and TEAD-signaling pathway in CAF-S1 decreases significantly in the stroma upon chemotherapy. Indeed, when we deactivate YAP1 in CAF-S1, the proprtion of T regs decrease, which suggest that YAP/TAZ TEAD pathway is implicated in the immunosuppression activity of CAF-S1. Subsequently, the inactivation of YAP1 in CAF-S1 increases significantly the cytotoxicity of CD8+ T cells measured by the expression/secretion of perforin and granzyme B in their surface. Interestingly, TEAD pathway is significantly downregulated upon chemotherapy in the stroma of PDX models. These data suggest that the YAP / TAZ pathway may be involved in the re-infiltration of lymphocytes upon treatment.

Results
Taken as a whole, our data show that YAP1 inhibition in CAF-S1 fibroblasts strongly reduces their immunosuppressive activity. These data thus highlight that the significant down-regulation of YAP1 and downstream TEAD-signaling pathway that we observed in CAF-S1 upon chemotherapy could be key in FOXP3+ T reg regulation and subsequent CD8+ T cell re-infiltration following chemotherapy.

MAINGUENÉ JULIETTE // Master 2
Responsable scientifique : BIECHE Yvan
Institut Curie, Unité de pharmacogénomique, Paris

HPV integration signatures and sites in head and neck squamous cell carcinoma

Context & Objectives

HPV prevalence in Head and Neck Squamous Cell Carcinoma (HNSCC) is ~26%. It primarily affects the oropharynx. HPV induced oncogenesis mainly involves viral onco-proteins E6 and E7 expression. In some cases, viral DNA integrates in the host genome. Integration into or near a cancer related gene can affect the gene expression and directly contribute to carcinogenesis.
In this study, we retrospectively assessed HPV integration sites and signatures in 80 HPV positive HNSCC, using the double Capture-HPV method followed by Next-Generation Sequencing.

Results

Patients had localized or locally advanced HPV positive HNSCC. HPV16 was the most frequently detected genotype (n=72, 90%). We confirmed the five previously described mechanistic signatures of HPV integration, i.e., episomal (EPI), integrated in a truncated form revealing two HPV-chromosomal junctions colinear (2J-COL) or nonlinear (2J-NL), multiple hybrid junctions clustering in a single chromosomal region (MJ-CL) or scattered over different chromosomal regions (MJ-SC). HPV remained as an episome or was integrated in the human genome in 31 (38.8%) or 49 (61.2%) cases respectively. The most frequent integration signature was MJ-SC (n=25, 31.3%). HPV integration signatures were not associated with patients' and tumors' characteristics or survival. As previously described in other HPV associated cancers, a low HPV copy number tended to be associated with a worse prognosis. We identified 267 HPV-human chromosome junction sequences scattered on most human chromosomes. Remarkably, among the integrated samples, we report four recurrent integration regions: PDL1, PDL2 and PLGRKT containing region (n=4/49, 8.2%), MYC and PVT1 region (n=3/49, 6.1%), MACROD2 (n=2/49, 4.1%) and KLF5/KLF12 region (n=2/49, 4.1%). PDL1 and MYC were overexpressed upon integration.

Conclusion & Perspectives

In conclusion, we identified recurrent targeting of several cancer related genes such as PDL1 and MYC upon HPV integration, suggesting a role for these genes in HNSCC carcinogenesis.

PRIX HÉLÈNE STARCK 2020

MENNOUR SABRINA // Doctorante
Responsable scientifique : VAGNER Stéphan
Institut Curie, UMR3348, Orsay

RNA binding activity of signalling proteins in melanoma

Context & Objectives

Recent studies have underscored the importance of RNAs in the regulation of protein-protein interactions. By allowing the assembly of protein complexes, non-coding RNAs act as scaffolds and thus promote protein-protein interactions in order to regulate the chromatin state. RNAs are also able to interact with proteins in order to modulate their activities, interactions or localisation. In the cytoplasm, highly regulated signalling pathways are regulated through a cascade of protein-protein interactions. In the MAPK (mitogen-activated protein kinases) signalling pathway, the binding of a ligand to a membrane receptor triggers a cascade of phosphorylation and protein-protein interactions that allow the transduction of the signal. Abnormal activity of this pathway through increased ligand binding or activating mutations lead to cellular dysfunction associated with tumor initiation and progression.
The potential role of RNAs in the direct regulation of protein-protein interactions of key cytoplasmic signal transduction pathways remains largely unknown. The aim of the thesis was to investigate and demonstrate the direct RNA binding activity of proteins involved in the MAPK pathway and to evaluate the role of RNA-protein interactions on intracellular signalling.

Methods

Using a combination of CLIP (crosslinking and immunoprecipitation) and silica matrix-based affinity capture (2C complex capture) approaches that can uncover direct interactions between proteins and RNAs in vivo, we demonstrated a direct interaction between key MAPK signalling proteins and RNA in melanoma cells. Subsequent microscopy studies using proximity ligation assay (PLA) led us to demonstrate an RNA-dependent modulation of protein-protein interactions in the MAPK pathway, suggesting that an RNA component is involved in the stabilization of these protein-protein interactions. We specifically identified a deletion mutant in BRAF, a central oncogenic protein and therapeutic target in melanoma, that lacks RNA binding activity and harbors decreased signaling activity.

Results

By highlighting the existence of an RNA-mediated modulation of protein-protein interactions, this study shows the unprecedented importance of the RNA binding activity of key signal transduction proteins that should be considered in the understanding and targeting of tumor cells.

ROUSSEAU MÉLANIE // Doctorante
Responsable scientifique : Le CAM Laurent
Institut de Recherche en Cancérologie de Montpellier, Equipe Oncogénèse Moléculaire

Role of E4F1 in melanocyte homeostasis and melanoma development

Context & Objectives
The nature of the metabolic networks involved in skin homeostasis is still poorly understood. However, previous work of our laboratory indicates that the multifunctional protein E4F1, an important regulator of the p53 tumour suppressor pathway, is a major player in skin homeostasis through its involvement in the regulation of the Bmi1-Arf-p53 pathway and the control of mitochondrial activity. The metabolic effects of E4F1 involve the control of pyruvate dehydrogenase (PDH), a mitochondrial enzyme that converts pyruvate into acetyl-CoA. Consistently, E4F1 inactivation in basal keratinocytes result in impaired PDH activity and in a metabolic reprogramming, redirecting the glycolytic flux towards lactate production. This leads to remodeling of their microenvironment and alterations of the basement membrane of the skin, inducing an exhaustion of the epidermal stem cell pool.

Methods
I further aim to investigate the consequences of pyruvate metabolism disruption in other skin cell types as the melanocyte lineage. Using different genetically engineered mouse models allowing the inactivation of E4f1 in these melanocyte cells, I showed for the first time that its inactivation leads to pigmentation defects independently of effects on their survival or differentiation. At the molecular level, these effects are linked to the transcriptional deregulation of MITF, a key player in pigmentation process. Finally, my data show that the disruption of pyruvate metabolism in melanoma cells influences their plasticity, a process that may contribute to their resistance to targeted therapies.

Conclusion & Perspectives
I now aim to refine our knowledge of the molecular mechanisms linking pyruvate metabolism to MITF regulation. These data should shed new light on the metabolic networks underlying melanocyte function and influencing melanoma progression.

PRIX HÉLÈNE STARCK 2020

SUAREZ GUADALUPE // Post-doctorante
Responsable scientifique : AMIGORENA Sebastian
Institut Curie, U932, Paris

Role of the epigenetic regulator Suv39h1 on CD8+ T cell tissue homing and resident memory differentiation

The immune system can influence cancer development and progression. While many tumours exhibit numerous mutational neoantigens, immune responses against tumours are still generally inefficient. The development of checkpoint blockade therapies, such as anti-PD-1, boost these T cell responses, demonstrating that immune responses can induce effective, long lasting, tumour rejection in cancer patients (1). A large proportion of T cells infiltrating tumours have a resident memory (Trm) phenotype (2). Trm differentiation in tumours, however, remains poorly understood. We showed recently that CD8+ T cells defective for the histone methyltransferase Suv39h1 show increased long-term memory differentiation (3), are more sensitive to anti-PD1 reprograming and more effective than WT cells in eradicating B16-F10 melanoma tumours (our unpublished results). Here, we show that the absence of Suv39h1 expression in CD8+ T cells promotes tissue homing and CD69+ Trm differentiation in infectious and non-infectious models, in a T cell intrinsic manner. The proportion of CD69+/CD103- cells was increased in Suv39h1 KO CD8+ T cells relative to control T cells, in lung tissue after 35 days of flu infection, suggesting that Trm differentiation is favoured in absence of Suv39h1 expression. In order to investigate tissue homing and Trm differentiation in a non-infectious model, we evaluated Trm formation after lymphopenia induced homeostatic proliferation. In this model too, we observed a 10-fold increase in homing of Suv39h1 KO CD8+ T cells to the lung, the skin, and the liver, as compared to WT cells. Suv39h1 KO Trm cells express high levels of CD49d (which is involved in tissue homing to inflamed sites). Single cell RNAseq analysis showed increased proportion of effectors among Suv39h1 KO cells in the blood, and an increase in CD69+ Trm cells in the lung tissue. Suv39h1 KO cells showed increased expression of the transcription factor Helios, which is involved in quiescent maintenance of autoreactive and regulatory cells. These results identify a new epigenetic regulator of Trm differentiation, and a potential target for manipulation of the pathway in the context of immunotherapy.

Publications related to the funded project
J. J. Havel, D. Chowell, T. A. Chan, (2019). *Nat Rev Cancer.*
2. A.-P. Ganesan et al., (2017). *Nat. Immunol.*
3. L. Pace et al. (2018)., *Science.*

JJC 2020

PRIX HÉLÈNE STARCK

SESSION 3

COMMUNICATIONS POSTERS

SALLE VIRTUELLE 3

MODÉRATEURS

Marianne BURBAGE - Julie PANNEQUIN - Claude PRIGENT

FOURNIE CLAIRE // Doctorante
Responsable scientifique : VERKINDT Chantal
Université de la Réunion, Le Tampon

Effects of a non-pharmacological intervention combining Heart Rate Variability Biofeedback and Adapted Physical Activity on post-treatment quality of life in adult hematologic patients.

Context & Objectives
In hematology, Adapted Physical Activity (APA) is recognized to improve physical functioning and fatigue, but questions remain about its impact on overall quality of life. Heart rate variability biofeedback (HRVB) is a behavioral intervention that have positive effects on emotional self-regulation. We propose to evaluate the effects of an intervention combining APA and HRVB on quality of life, fatigue, anxiety, and depression in adult patients in remitted of hematologic malignancies, as part of the APACCHE (Adapted Physical Activity and Cardiac Coherence in Hematologic Patients) protocol.

Method
Patients were randomly assigned into two 12-week treatment groups: experimental arm (HRVB + APA) versus control arm (APA alone). APA included aerobic and resistance training for 1-h twice weekly; HRVB consisted of 10 supervised sessions and daily home-based practice of rhythmic breathing at a frequency close to 6 cycles/min using biofeedback of heart rate variability monitored with a pulse sensor. Questionnaires were administered to assess quality of life (SF-36), fatigue (MFI-20), and anxiety and depression (HADS); initially (T1), at 6 weeks (T2), at 12 weeks (T3) and at 24 weeks follow-up (T4). We have also used a qualitative method with semi-structured interviews conducted in 12 patients at T4.

Results
Forty patients in remission (yr 53.5 ± 14.2) were included. Among them, 7 gave up (5 control arm, 3 experimental arm); 1 was excluded due to absenteeism >50%; and 32 was included in per protocol analysis. At T3, we observed improvement in all SF-36 subscales; with the exception of the «body pain» and «general health» subscales. Especially, the control arm has a greater improvement in "mental health" and in mental component summary score. We measured decrease of "General and Physical Fatigue» and «Mental Fatigue» subscales of MFI-20; and of anxiety and depression scores of HADS. The semi-directive interviews were conducted in 12 patients and provided additional information about the patient's experience. The qualitative analysis based on the transcripts allowed to better understand the changes in patients and to analyze the differentiated effects of our interventions on quality of life.

Conclusion
Although the contradictory results of the questionnaires, the qualitative method highlighted the complementarity between APA and HRVB; the added value of HRVB; as well as their combined effectiveness in improving post-treatment quality of life.

Publication related to the funded project
Fournie C. *Pilot study: first results of an adapted physical activity program in hematologic patients. In An. of Be. Med.*
Fournié C. *Adapted Physical Activity and Cardiac Coherence in Hematologic Patients (APACCHE): Study Protocol for a Randomized Controlled Trial. BMC Sports Sc., Med. and Re.*
Fournié C. *Heart rate variability biofeedback for reduction of symptomatology in chronic diseases: a systematic review. In: Comp. Th. in Med.* (submitted)

GIMENEZ ANDRES MANUEL // Doctorant
Responsable scientifique : COPIC Alenka
Institut Jacques Monod, Paris

The amphipathic helix of perilipin 4, but not of other perilipins, can stably coat lipid droplets

Context and objectives
Lipid droplets (LDs) are organelles with a central role in lipid metabolism by storing energy in the form of neutral lipids. The number and size of LDs vary depending on cell type and their physiological state. Many types of cancer cells display an increase in LD content (including breast, liver, lung and pancreatic cancers). We are interested in how cytosolic proteins interact with LDs and control their stability and size. Many LD proteins contain amphipathic helices (AHs), structural motives that can interact directly with membranes. Perilipins (Plins) are abundant LD proteins that contain AHs and that regulate LD metabolism. This works aims to explore if Plin AHs coat and stabilize LDs.

Methods
We study how AHs from human Plins interact with LDs using cellular and biochemical reconstitution approaches. We use purified Plin AHs in biochemical reconstitution assays to observe their interaction with neutral lipids and test their dynamics with a microfluidics set-up that we developed. Moreover, we expressed AHs from human Plins as fluorescent fusion proteins in budding yeast to compare their LD targeting capacity and their effect on LD stability.

Results
The purified AH of one Plin member, Plin4, can strongly interact with neutral lipids in the absence of any phospholipids. Plin4 AH forms small oil particles that remain remarkably stable over a period of many weeks. Plin4 AHs form an immobile layer on the oil surface. Purified perilipin 3 (Plin3) AH can also form oil particles; however, these particles are less stable over time and Plin3 AH exchanges between the oil surface and the solution, in contrast to Plin4 AH. When expressed in budding yeast, all Plin AHs can target LDs. However, Plin4 AH interacts very stably with LDs in the yeast model, whereas the binding of other Plin AHs is much more dynamic. Remarkably, LDs coated by Plin4 AH in yeast are smaller than the ones coated by other Plins. Using Plin4 AH mutants, we show that the conservation of polar residues and their positioning along Plin4 AH contribute to its stable interaction with lipids. We propose that the polar residues of Plin4 AH mediate a multimeric structure that forms a coat on LDs, thereby increasing their stability and decreasing LD size. In contrast, other Plin AHs interact with LDs as monomers.

Conclusion
In conclusion, we propose that Plin4 AH forms a very stable and immobile coat on LDs. Plin4 AH would stabilize LDs and reduce their size.

Publications related to the funded project
Giménez-Andrés, M., Emeršic, et al. (2020). *BioRxiv.* (Under revision in Elife)
Copic, A., Antoine-Bally, S.*, Giménez-Andrés, M.*, et al. (2018). *Nature Communications.*

HAMANN PIERRE // Master 2
Responsable scientifique : ROBERT Caroline
Gustave Roussy, équipe Prédicteurs moléculaires et nouvelles cibles en oncologie, U981, Villejuif

Role of initiation complex eIF4F in immune checkpoint therapy resistance

Context & objectives

Although immunotherapy has revolutionized the management of melanoma, some patients do not respond to this treatment and others develop secondary resistance. It is therefore necessary to better understand the resistance mechanisms of immunotherapies in order to propose better alternatives therapeutic. Our project aims to study the translational landscape and the role of the eIF4F translation initiation complex. Our preliminary results show that there is an increase in the eIF4F complex and translation during the development of resistance. We wish to identify which mRNAs have involved during this process.

Results

We have identified a group of mRNAs which translation are dependent of eIF4F in two anti-PD1-resistant melanoma cell lines. Then, we validated this transcriptional signature and correlated it to the increased translation of these mRNAs by performing several polysome profiling experiments. However, treatment of these cells with an eIF4F inhibitor shows a heterogeneous translation inhibition of all our mRNA in this model. We then confirmed in vivo in mouse tumors that eIF4F inhibition appears to increase the response to antiPD1 in vivo.

Conclusion & perspectives

Our preliminary results suggest that silvestrol may improve the therapeutic response to anti-PD1 in vivo. These results will need to be confirmed in other resistant melanoma cell lines and in other cancer models. In addition, we would like to identify these targets by immunohistochemistry on mouse and human sample cohorts.

PRIX HÉLÈNE STARCK 2020

LOZANO ANTHONY // Doctorant
Responsable scientifique : HIBNER Ursula
Institut de Génétique Moléculaire de Montpellier, UMR5535

Impact of Ras signaling on subclonal heterogeneity in hepatocellular carcinoma

Context and objectives

Hepatocellular carcinoma (HCC) is a heterogeneous disease with poor clinical outcomes. Identification of common oncogenic events driving HCC development allowed molecular and physiopathological classifications of the tumors. In addition, deregulation of some pathways spans different HCC types. For example, MAPK Erk signaling, which is associated with poorly differentiated, aggressive tumors, is detected in over 50% of HCC. In other cellular contexts, it has been shown that quantitative differences in MAPK signal intensity affect cellular phenotype and fitness. We hypothesized that variations in the level of activation of the pathway might trigger selective forces that would generate a novel type of intratumoral heterogeneity that we called quantitative heterogeneity. In order to study population dynamics and intercellular interactions, we developed a murine model of HCC that combines intrahepatic injection of cells and lineage tracing. To model the activation of MAPK we used hepatic progenitors to generate transformed cells expressing a constitutively active form of the Ras oncogene -HRasG12V. In our experimental set-up, the expression level of Ras is strictly correlated with the fluorescence intensity of reporter proteins (Venus or mCherry).

Results

After cell sorting, we have obtained populations with defined levels of RasG12V expression that resulted in defined mean levels of MAPK Erk activation. The Ras-HIGH and Ras-LOW cellular populations were co-injectected as orthotopic xenografts and their fate was followed through fluorescence tracing. We showed that while the ex vivo cell growth is only marginally affected by a wide range of RasG12V levels, for in vivo tumorigenesis there exists a narrowly defined optimal level of the oncogene expression. Strikingly, the selective advantage afforded by the optimum Ras/MAPK signaling intensity depends on the tumor microenvironment, which differs between the primary and metastatic tumors. RNAseq and flow cytometry-based analyses of the tumoral and stromal components highlighted the role of M2-type macrophages and dendritic cells in shaping the selective tumor microenvironments.

Conclusions & perspectives

Overall our data defined a hepatocyte-specific MAPK activation signature and identified an Erk target, involved in the tumor-stroma interactions which likely gives rise to a site-specific selective advantage for tumor cells characterized by a defined MAPK Erk signal intensity.

POTY SOPHIE // Post-doctorante
Responsable scientifique : POUGET Jean-Pierre
Institut de Recherche en Cancérologie de Montpellier

Comparison of radiolabeled anti-EGFR IgG and F(ab')2 for the evaluation of EGFR expression in mouse models of pancreatic dutcal adenocarcinoma

Context & Objectives

Panitumumab is an anti-Epidermal Growth Factor Receptor (EGFR) monoclonal antibody FDA approved in 2006 for the treatment of EGFR+ metastatic colorectal cancer. Panitumumab was previously radiolabeled with 89Zr/111In and its potential as a PET/SPECT tracer was demonstrated in colorectal, skin and ovarian carcinomas mouse models. Pancreatic ductal adenocarcinoma (PDAC) currently lacks non-invasive diagnostic tools and EGFR is expressed in >55% of patient samples. [89Zr]Zr-DFO-panitumumab and its F(ab')2 fragments are investigated for the evaluation of EGFR expression in PDAC mouse models. The impact of the radioimmunoconjugate size is evaluated in terms of biodistrubution and pharmacokinetic profiles.

Results

Immunohistochemistry revealed strong EGFR expression in a broad range of PDAC tumors including AsPC1, BxPC3 and Suit-2. Panitumumab and its F(ab')2 fragment were functionalized with the DFO chelator and radiolabeled with 89Zr. Radiolabeling with 89Zr yielded the desired radioimmunoconjugate with excellent radiochemical yield (>99%) and good specific activity (220 MBq/mg). Cell binding assay on AsPC-1, BxPC3 and Suit-2 cell revealed internalization of the radioconjugates (2.9-15.6% of added radioactivity) and membranous retention (22.3-55.9% of added radioactivity). [89Zr]Zr-DFO-panitumumab/F(ab')2 (11.0 MBq, 50 µg) was intravenously injected in mice bearing subcutaneous/orthotopic EGFR+ xenografts (e.g. BxPC3, AsPC-1, Suit-2). PET images showed a strong and persisting accumulation of the radioimmunoconjugates at the tumor with high tumor-to-normal tissue contrast. Volume of interest analysis and biodistribution studies confirmed the high tumor uptake (10.7 ± 1.6%ID/g for [89Zr]Zr-DFO-panitumumab versus 8.1 ± 0.5%ID/g for [89Zr]Zr-DFO-F(ab')2 in BxPC3) at 24 h post-injection. Blocking study with co-injection of excess panitumumab (300 µg), resulted in a consistent decreased tumor uptake and validated the specificity of the radioconjugate for EGFR.

Conclusions

Our study highlights the potential of [89Zr]Zr-DFO-panitumumab/F(ab')2 as an immuno-PET tracer for the noninvasive evaluation of EGFR expression in PDAC. Such approaches are critical to guide therapy selection and optimal dose finding. Panitumumab EGFR affinity should be evaluated in a theranostic approach with therapeutic isotopes for targeted radionuclide therapy.

PRIX HÉLÈNE STARCK 2020

MIRO PINA CARIDAD // Doctorante
Responsable scientifique : DUHARCOURT Sandra
Institut Jacques Monod, Paris

Identification of a novel protein associated with Polycomb Repressive Complex 2 in Paramecium

Context & Objectives

Epigenetic aberrations caused by a change of chromatin structure, such as histone modifications, lead to heritable alterations of gene expression and promote tumorigenesis. Trimethylation of lysine 27 on histone H3 (H3K27me3) mediated by the Polycomb Repressive Complex 2 (PRC2) plays a critical role in regulating gene expression. Deciphering the mechanisms that target PRC2 across the genome is one important challenge today. We use the unicellular eukaryote Paramecium as a model organism to unravel these mechanisms. In this system, newly established chromatin regions with Polycomb signature are physically eliminated during development of the somatic genome. Therefore, the initial establishment of PRC2 is not blurred by the maintenance mechanisms. The aim of my project was to unravel how Paramecium PRC2 complex is targeted to specific regions of the genome.

Results

In order to gain insight into the mechanisms that mediate Paramecium PRC2 complex recruitment to chromatin, I identified the proteins that are associated with its catalytic subunit (Ezl1). To do so, we pulled down a tagged version of Ezl1 by tandem affinity purification using Paramecium nuclear extracts. Mass spectrometry analysis allowed us to identify several Ezl1-associated proteins that were uniquely found within tagged-Ezl1 preparations and not in the control. We have thus identified the putative Paramecium PRC2 core complex and an uncharacterized protein (Eap-1) with no conserved domain. The reverse immunoprecipitation using Eap-1 as bait confirmed the interactions with Ezl1. ChIP-seq experiments further showed that H3K27me3 is not correctly targeted upon Eap-1 depletion.

Conclusion & Perspectives

Altogether, our data showed that Eap-1 is essential for PRC2-Ezl1 targeting. In order to understand how Eap-1 guides PRC2-Ezl1 recruitment to chromatin, I will perform a tagged-Ezl1 pull down from Paramecium nuclear extracts in cells depleted for Eap-1.

VAN BODEGRAVEN EMMA // Post-doctorante
Responsable scientifique : ETIENNE-MANNEVILLE Sandrine
Institut Pasteur, équipe Migration et Cancer, Paris

Mechanics of glioblastoma cells during migration and invasion

Context & Objectives
Glioblastoma multiforme (GBM) is the most common malignant brain tumor with a poor prognosis and no curative therapy available. GBM invasion largely contributes to the poor prognosis and therapeutic failure. Knowledge on the mechanism of GBM cell invasion is essential to develop new therapies. Recent literature points towards the mechanical properties of tumor cells and the extracellular matrix as essential players in GBM invasion. Because of their inherent physical properties and crosstalk with other cytoskeletal element intermediate filaments (IFs) are ideal candidates to modulate cell mechanics. This study aims to determine the contribution of IFs in the mechanics of GBM cells and in GBM invasion.

Results
Glial and GBM cells express the IF proteins GFAP, Vimentin, Nestin and Synemin at different levels. The intratumoral heterogeneity of GBMs is likely to be reflected by a large diversity in the expression of these IF genes. To map the heterogeneity of IF expression in GBMs, we have analyzed published single-cell RNA sequencing data generated from patient material. Hierarchical clustering of single GBM cells based on IF gene expression identified 12 different cell clusters and showed that high IF gene expression signatures associate with markers of cells localized at the tumor periphery. We further observed a strong correlation between high IF gene expression and cell migration and mechanosensing gene ontology clusters.

Methods
Using CRISPR-Cas9 technology to alter IF gene expression in GBM cell models we have recapitulated this association between IF expression and GBM invasion-related gene expression in patients. Furthermore, in 3D in vitro and in vivo migration assays we have observed that the loss of IF expression decreases the ability of GBM cells to invade as leaders and impairs in vivo invasion in the zebrafish brain.

Conclusion & Perspectives
Our results show that IF gene expression is associated with GBM invasion in tumors of patients and that IFs are important for GBM invasion in in vitro and in vivo assays. In our current studies we use 2D and 3D migration assays on different rigidities and within confined environments to further unravel how IFs modulate cell mechanics to promote GBM invasion.

PRIX HÉLÈNE STARCK 2020

VOISIN ALLISON // Post-doctorante
Responsable scientifique : GRINBERG-BLEYER Yenkel
Centre de Recherche en Cancérologie de Lyon

Understanding the roles of NF-κB subunits on effector CD8+ T cell functions and response to anti-PD-1 checkpoint blockade therapy in melanoma.

Context & Objectives
Cancer progression is greatly influenced by a delicate balance between immunity and tolerance to tumors. The recent success of immunotherapies, such as immune checkpoint blockers, demonstrates that this balance can be shifted effectively towards enhanced anti-tumor immunity, to delay or even stop cancer progression. In particular, CD8+ effector T lymphocytes (Teff) play a central role in the elimination of tumors. Understanding the molecular mechanisms that drive these responses therefore has major implications for the development of improved therapies. Our recent reports demonstrated that the transcription factor Nuclear Factor Kappa-light-chain-enhancer of activated B cells (NF-κB) is a critical modulator of immunity and tolerance to cancer. However, NF-κB is in fact a family of transcription factors composed of 5 distinct subunits and the contribution of individual subunits to murine and human CD8+ T-cell biology has never been addressed. In this project, we are using CRISPR-Cas9 approaches and unique mouse models to investigate the selective contributions of NF-κB subunits to CD8+ T-cell function and response to anti-PD-1 therapy.

Results
In a first set of experiments, we investigated the role of RelA and c-Rel in primary human CD8+ T cells in vitro. CRISPR-Cas9-mediated ablation of either gene led to defects in proliferation and cytokine production. Interestingly, the effects of RelA and c-Rel-deficiency on cytokines production were distinct, suggesting that these subunits control selective T-cell functions. Consistent with this hypothesis, our transcriptome analysis highlighted specific gene expression patterns controlled by RelA and c-Rel.

Conclusion & Perspectives
Our experiments delineated for the first time the involvement of discrete NF-κB subunits in the control of human CD8+ T cells. We are now in the process of analyzing mouse models carrying specific ablation of RelA and c-Rel in CD8+ T cells, in the steady-state and in response to anti-PD-1 checkpoint blockade therapy in melanoma. These studies may provide a mechanistic understanding of the efficacy of checkpoint-blockade therapies and reveal specific NF-κB subunits activities that can be targeted by novel therapeutics to enhance effector T cell function.

Publications related to the funded project
Grinberg-Bleyer, Y., Caron, R., Seeley, J.J., Silva, N.S.D., Schindler, C.W., Hayden, M.S., Klein, U., Ghosh, S. (2018). *The Alternative NF-kB Pathway in Regulatory T Cell Homeostasis and Suppressive Function. The Journal of Immunology.*

Grinberg-Bleyer, Y., Oh, H., Desrichard, A., Bhatt, D.M., Caron, R., Chan, T.A., Schmid, R.M., Klein, U., Hayden, M.S., Ghosh, S. (2017). *NF-kB c-Rel Is Crucial for the Regulatory T Cell Immune Checkpoint in Cancer. Cell.*

JJC 2020

PRIX HÉLÈNE STARCK

SESSION 3

COMMUNICATIONS POSTERS

SALLE VIRTUELLE 4

MODÉRATEURS

Bernard DE MASSY - Marie-Caroline DIEU-NOSJEAN

ABOUELMAATY MOHAMED // Post-doctorant

Responsable scientifique : METZGER Daniel
Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch-Graffenstaden

Preclinical evaluation of the therapeutic potential of a novel vitamin D analog in prostate cancer treatment.

Context & Objectives

Vitamin D based therapies have been proposed in the prevention and treatment of various forms of cancer, including prostate cancer. However, studies investigating this potential have either used sub-pharmacological doses of vitamin D in humans to avoid hypercalcemia, or have not characterized the putative anti-tumor effects in disease-relevant experimental models. To comprehensively examine the effects of a hypocalcemic vitamin D analog in prostatic intraepithelial neoplasia (PIN), genetically-engineered mouse models of prostate cancer were treated with the analog, and the responses of the various cell types present were characterized using single-cell RNA-sequencing.

Results

We show that the analog induces apoptosis in senescent PINs in an epithelial vitamin D receptor (Vdr)-dependent manner, but also activates anti-apoptotic mechanisms in a specific epithelial subset. Furthermore, the analog normalizes extracellular matrix remodeling by cancer-associated fibroblasts, and reduces the prostatic infiltration of immunosuppressive myeloid-derived suppressor cells in an epithelial Vdr-independent manner.

Conclusion & Persectives

Findings of this characterization delineate the distinct responses of prostatic precancerous lesions and the microenvironment to the vitamin D analog.

PRIX HÉLÈNE STARCK 2020

BEKKAT FÉRIEL // Doctorante

Responsable scientifique : PREVOST BLONDEL Armelle
Institut Cochin, Inserm U1016 - CNRS UMR8104, Paris

Rôle pro-tumoral de l'IL4 induced gene 1 (IL4I1) dans le contexte du mélanome

Context & Objectives

Human cutaneous melanoma is the archetypal immunological cancer with a high density of tumor-infiltrating lymphocytes. In contrast to the role of T cells in immunosurveillance, that of B cells has been poorly investigated in melanoma, whereas both conventional (B2) and innate-like (B1) B cells are present in skin at homeostasis and during inflammation.

Methods

Our project aims to explore B1 and B2 roles in tumor progression and to understand the molecular mechanisms underlying.
Here, we show that B cell depletion delays the metastatic dissemination in RET mice, a spontaneous model of melanoma, and is associated with impaired CD4+ and CD8+ T cell activation. Whereas B2 cells constitute the predominant B cell subset in the primary tumor at early stage, B1 cell proportions progressively increase. Thus, B2 cells might exert a protective role in RET mice.

Results

Our recent data established that the genetic inactivation of the immunosuppressive enzyme, IL4-induced gene 1 (IL4I1), delays tumor cell dissemination and is associated with an increased number of tumor-associated B cells in RET mice. Regarding the key role of IL4I1 in the physiological differentiation and response of mouse B2 cells, its inactivation might potentiate the protective role of B2 cells in melanoma. We are currently investigating how IL4I1 affects B cell recruitment and functions in melanoma to give a more comprehensive view of its pro-tumoral effects.

Conclusion & Persectives

Immunotherapy with immune checkpoint inhibitors targeting programmed death 1 has revolutionized the treatment of metastatic melanoma. Nevertheless, 60% of treated patients resist to current treatment. Our data could strengthen the rationale for targeting of IL4I1, thus stimulating anti-tumoral B cells, in combination with current immunotherapy in patients with metastatic melanoma.

LIU JING // Doctorante
Responsable scientifique : RADVANYI François
Institut Curie, UMR144, Paris

Molecular characterization of retinoblastoma by bioinformatic analysis of genomic, transcriptomic and methylomic data

Context & Objectives
Retinoblastoma is a rare childhood cancer of the developing retina. With timely diagnosis, it can be treated by chemotherapy, but in some cases, enucleation of the eye is still needed to cure the disease. If treated too late, it may lead to metastasis and death.
Bi-allelic inactivation of the retinoblastoma gene (RB1) is the main genetic lesion that in most cases initiates retinoblastoma. Other genetic and/or epigenetic alterations maybe needed for tumorigenesis, but are still unclear.

Methods
The human retina consists of seven types of cells: rod and cone photoreceptors, bipolar, amacrine, horizontal, ganglion cells, and Müller glia, all differentiated from retinal progenitors. Cone cells were proposed to be the cell of origin by several studies, but controversy exists.
The objective of our study is to have a thorough molecular characterization of retinoblastoma, to look for possible subtypes of the disease, to identify further genomic and epigenetic alterations beyond RB1 mutations, and to characterize the molecular pathways involved in tumorigenesis.

Results
Through studying genomic, transcriptomic, and methylomic data on a series of 102 retinoblastomas, we identified two molecular subtypes of retinoblastoma with distinct clinical, pathological and genetic features. Subtype 1 retinoblastomas presented earlier onsets, more bilateral and heritable cases, and most often exophytic growth. Subtype 2 retinoblastomas presented mainly endophytic growth, with higher genomic instability and higher inter- and intra-tumor heterogeneity. All retinoblastoma expressed early cone cell markers, of subtype 1 tumors expressed more mature cone signatures and subtype 2 expressed less differentiated ones. As there were no metastatic cases in our initial series of retinoblastoma, we also showed that subtype 2 tumors were associated with higher risks of metastasis by an independent series of 112 retinoblastoma from southern America.

Conclusion & Perspectives
The characterization of these two subtypes has important implications for retinoblastoma biology, diagnosis, management and the development of more specific treatments."

PRIX HÉLÈNE STARCK 2020

PARENT PAULINE // Master 2
Responsable scientifique : DUTERQUE-COQUILLAUD Martine
Institut Pasteur de Lille, équipe Target, UMR 9020 CNRS - UMR-S 1277

FSCN1 expression and its role in neuroendocrine prostate cancer

Context & Objectives
Prostate cancer is the second leading cause of cancer-related death. Despite the initial androgen deprivation therapy (ADT) efficacy, nearly all patients evolve to castration resistance prostate cancer (CRPC), and a small proportion of them then acquires a neuroendocrine phenotype (NE) with a neuronal marker expression that includes either chromogranin A (CgA) or neuro-specific enolase (NSE). Neuroendocrine prostate cancer (NEPC) is the result of various heterogenous molecular mechanisms, but, generally, the expression and activity of the androgen receptor (AR) are lower. Our recent in vitro studies showed that the Fascine 1 gene (FSCN1) is repressed by AR in androgen-dependent cell lines. Whilst absent in normal epithelial tissues, FSCN1 is associated with the progression of many cancers such as ovarian or colon cancer, and is indispensable to invadopodia formation; all of which take shape during the migration and invasion of cancer cells. This study aims to evaluate the expression and role of FSCN1 in NEPC as its incidence is increasing and is often associated with poor prognosis.

Results
First, we established an NE cell model based on two AR-dependent prostate cancer cell lines, LNCaP and VCaP. We performed two different procedures: a culture with a hormone-free medium for 14 days and a treatment with a second-generation ADT (enzalutamide) for 72 hours. Under these conditions, the expression of prostate-specific antigen, an AR-dependent gene, is decreased, which commonly indicates a loss in AR activity. The NE markers, both CgA and NSE, appeared, thus validating the model. We then observed increasing FSCN1 expression in both transcriptional and protein analyses. Migration and invasion were promoted in our model. We found out that when the FSCN1 gene is silenced with siRNA, migration and invasion were reduced. Second, the in silico analysis of transcriptomic data from cohorts published in cBioportal® showed a high expression of FSCN1 in the NEPC samples. Finally, using immunochemistry analyses on human samples, while FSCN1 expression was absent in primary prostate adenocarcinoma, we detected this protein in NEPC (6 samples /7).

Conclusion & Perspectives
The FSCN1 gene is expressed in NEPC and repressed by AR, which might contribute to its aggressiveness. Therefore, together with other genes, FSCN1 could belong to a specific molecular signature of NEPC occurrence. The next challenge will be to detect FSCN1 in liquid-biopsy.

PINTO GIULIA // Doctorante
Responsable scientifique : BROU Christel
Institut Pasteur, Paris

Role of Tunneling Nanotubes in Glioblastoma treatment-resistance

Glioblastoma (GBM) is the most aggressive and deadly of the primary brain tumours, as it is able to relapse despite surgery, chemo and radiotherapy. The mechanism of resistance to treatment is not fully understood, but its recurrence appears to be due to the presence of GBM stem cells (GSCs). Tunneling Nanotubes (TNTs) are thin open membrane connections that allow the cytoplasmic continuity of two distant cells and the bidirectional transfer of cellular material. TNTs play an important role during development, in the dissemination of viruses and in several neurodegenerative diseases. TNTs are also implicated in cancers where their presence and functionality have been correlated with tumour progression. Recent data have shown that in GBM, GSCs are interconnected in a vast network through thick neurite-like protrusions called Tumor Microtubes (TMs), allowing the propagation of ion flows through GAP-like Junctions. The extent of this network has been correlated with high resistance to treatment as well as cell invasion. One of my main objectives was to determine whether, in addition to the TMs, connections corresponding to the functional definition of TNTs, thus allowing the transfer of cellular material, existed in the GBM models and whether there was a correlation between their presence and the tumour phenotype or its resistance to treatment. To this aim, in my research project, I studied TNT-mediated communication in three GBM cell lines and in two patient-derived GSCs obtained from distinct areas of the same tumor. GBM cell lines can form TNTs whose functionality has been evaluated by quantifying the transfer of vesicles and mitochondria by imaging and flow cytometry. GSCs also form TNTs when grown in adherent culture but also in three-dimensional tumour organoids, a model that better summarises the characteristics of the tumour. Of interest, the two GSCs showed different TNT communication capabilities, in both control and irradiated conditions, with higher TNT activity (and mitochondrial transfer) in cells from the area with a high potential for relapse, as clinically characterised by functional magnetic resonance imaging. In the organoid model, I observed that the GSCs are interconnected in a network composed of both TMs and TNTs. In conclusion, I propose that TNTs exist in GBM tumour networks, where they allow the transfer of cellular material and that together with TMs they are involved in the resistance to treatment and the relapse of tumours.

Publications related to the funded project

Giulia Pinto, Christel Brou, et al. (2020): *The Fuel of Tumor Progression?,Trends in Cancer.*

VIAL ANTHONY // Doctorant
Responsable scientifique : MILHIET Pierre-Emmanuel
Centre de biochimie structurale, Montpellier

Molecular determinants and membrane mechanics involved in Nuclear Pore Complex neogenesis

Context & Objectives

Proliferating cells have to duplicate their constituents during interphase. While DNA replication is widely studied, duplication of other materials is largely unexplored. In particular, Nuclear Pore Complex (NPC) replication is a complicated process combining macromolecular assembly and membrane remodelling. Indeed, the two membranes composing the nuclear envelope must fuse locally. Moreover, the nuclear permeability barrier must remain intact during the whole process, underlining that protein assembly and membrane fusion must be tightly coordinated.
Importantly, NPCs are not renewed in non-dividing (post-mitotic) tissues . Thus NPC assembly specifically occurs in dividing cells and appears as an interesting target to specifically impair cancer cell growth, with low toxicity against non-proliferative tissues. In this context, my project aims at understanding the molecular mechanisms of NPC assembly, and in particular how membrane remodelling and protein recruitment are coordinated in space and time. To do so, I have combined super-resolution fluorescence microscopy with Atomic Force Microscopy (AFM) to get the topography of the nuclear membranes and nuclear pores, either fully assembled or assembly intermediates.

Results

We obtained the first AFM images of the nucleoplasmic side of human NPCs. Nanometer scale topography of the inner nuclear membrane gives information on membrane deformation and 3D structure of pore intermediates and we identified several types of structures: (i) mostly fully assembled pores exhibiting a ring of about 80nm diameter (at the ridge) and 30 nm height, similar to Xenopus laevis NPCs, (ii) pore intermediates exhibiting very diverse structures, from a central core to ring-like structure lacking of the NPC basket sub-complex. These structures correspond to different stages of NPC assembly and the combination with super-resolution allowed us to describe in more details the steps of NPC interphase assembly.

Conclusion & Perspectives

Our work gives a comprehensive view of the interphase NPC assembly. Very recent work has shown that blocking NPC formation selectively induces cancer cell death. These advances might in the future give another angle to tackle cancer.

ZHOU YA // Post-doctorante
Responsable scientifique : VIOVY Jean-Louis
Institut Curie, Laboratoire Physico-Chimie Curie, UMR 168, Paris

Droplet-based Textile Microfluidics for High-Throughput Anticancer Drug Screening

Context & Objectives

Advances in genomics and cell biology motivate the development of better targeting in therapeutical strategies. However, the limited size of biopsy samples, the long process to establish animal models, and the inefficiency of in vitro testing protocols, all makes the patient-specific drug screening costly and impractical. In this project, we try to tackle these problems by downscaling the drug testing assay, using patient-derived tumoroids cultured in a new experimental platform, droplet textile microfluidics, to shorten the drug testing period and reduce the sample consumption and cost per assay.

The generation of tumoroids is powered by the droplet microfluidics approach, in which each droplet can be regarded as an independent "microassay". A single 3D tumoroid (diameter > 200 µm), cultured from 200 cells, is formed in each droplet. The cellular apoptosis assay can be performed within these tumoroids droplets. The consumption of cells and reagents is reduced 20-100 folds per data point as compared to microplate assays. Using cells dissected from one PDX mouse, 50 drug combinations with 10 concentrations can be tested. To enable a drug screening protocol similar to the microplate assay, based on resazurin, we are developing a programmable droplet merging technique. It will achieve a sequential addition of hundreds of µl drug and resazurin to the tumoroid droplet. The entire protocol is performed in microchannels in a completely automated manner.

Methods

In order to keep cost compatible with large-scale screening, we shall apply for the fabrication of the microfluidic chips an innovative microfabrication method using textile technologies, Free-Flow Textile chips (FFTC). The FFTC is fabricated by industrial weaving process, with microchannels defined by sacrificial fibers on the woven textile. The textile is then impregnated with a gas-permeable matrix. The sacrificial wires are then removed, leaving well-defined cylindrical microchannels, in which "trains" of droplets are injected and manipulated.

In parallel, we are building a low-cost, open resource instrument prototype, comprising a motorized XYZ translocation stage, a multi-channel syringe pump for droplet pipetting, and a LED-based optical system for visualization and and "on flight" fluorescent imaging quantification. All are controlled by a single board microcontroller.

JJC 2020

PRIX HÉLÈNE STARCK

SESSION 3

COMMUNICATIONS POSTERS

SALLE VIRTUELLE 5

MODÉRATEURS
Michaela FONTENAY - Nicolas PENEL

BASBOUS SARA // Post-doctorante
Responsable scientifique : MOREAU Violaine
Université de Bordeaux

Silencing of Rnd3/RhoE blocks the growth of human hepatocellular carcinoma cells through the induction of senescence

Previously, we identified the Rho GTPase member family, RND3 as a metastasis suppressor gene in hepatocellular carcinoma (HCC). Indeed, RND3 expression was significantly lower in invasive HCC samples with satellite nodules. Here, we show that despite the favoring effect on invasion, Rnd3 knockdown induces a cell growth arrest not only in vitro but also in vivo in subcutaneous xenograft performed in mice. We did not detect any modification of the percentage of apoptotic/necrotic positive cells after Rnd3 inhibition in the HCC cell lines. Proteomics and molecular analysis revealed that the expression of CDK2 involved in cell cycle was altered in Rnd3-depleted cells. Interestingly, Rnd3 silencing significantly prevented the growth of HCC cell lines by inducing cellular senescence in a P16/Rb-independent manner. We further identified the loss of PTEN after Rnd3 inhibition as a crucial event for the development of senescence. Finally, our results demonstrate that the senescent cells exhibit an invasive ability suggesting that senescence could participate in metastasis.

GOUNGOUNGA JUSTE // Post-doctorant
Responsable scientifique : JOOSTE Valérie, BOUSSARI Olayidé
Université de Bourgogne, équipe Epidémiologie des Cancers Digestifs, UMR 1231, Dijon.

Correcting expected mortality of cancer patients in a cure model to better estimate the time to cure: empirical comparison using testicular cancer data.

Context & Objectives

Cure models have long been used in population-based studies to estimate the proportion of people "statistically cured" from a given cancer, i.e. the proportion of people diagnosed with the studied cancer who do not die from it. In the context of 'right to be forgotten' and access to insurance, there is a need for estimating the time to cure as the delay between diagnosis and the time from which statistical cure is reached. Estimating the time to cure can be used to improve access to insurance for cancer survivors. Boussari et al. proposed a model for estimating this indicator, based on the fact that observed mortality is the sum of two forces of mortality attributable to cancer (excess mortality) and other causes of death respectively. Usually, a proxy for mortality by other causes is drawn from the general population life tables. We propose adapting this model by correcting the mortality by other causes in situations where the life table is not adapted and therefore excess mortality is incorrectly estimated.

Methods

Boussari et al. proposed a cure model, based on a modified Beta function, allowing to estimate, the time from which the excess mortality becomes null. Like other models of excess mortality, this approach assumes that the expected mortality of the studied patients is comparable to that of the general population. Several studies indicate that this might not be the case. To account for this non-comparability, we have introduced a scale parameter in this model correcting the expected mortality of patients. We empirically compared the performance of this model to the one proposed by Boussari et al. from testicular cancer.

Results

Applied to testicular cancer data, we were able to estimate a time to cure in the middle-aged individual (62 years) at {proposed model: (95% CI95% = 4,59 [4,58; 4,60]) vs baseline model: 3,74 years (95% CI95% = [3.73; 3.75] years)} after diagnosis and a non-comparability effect at {proposed model: 0,69 (95% CI95% = [0,59; 0,80]) }, an AIC equal to {proposed model: 849,72 vs baseline model: 2768,353).

Conclusions & perspectives

We recommend the proposed model for the estimation of the time to cure in situations where it is suspected that the expected mortality of patients does not correspond to that given by the available life tables. This model is implemented in an R package that we plan to make available soon to disseminate its use in the analysis of cancer registry data.

LANDWERLIN PAULINE // Doctorante
Responsable scientifique : ROMIER Christophe
Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch

Study of the SMC1/SMC3/RAD21/STAG1-2 complex of human Cohesin and its implication in cancer

Context & Objectives

Chromatin structure and epigenetic effectors regulate most nuclear processes. Impairment of epigenetic mechanisms is responsible of a large set of diseases, particularly in the onset and progression of cancers. The Cohesin complex is an essential epigenetic player involved in chromosome cohesion, DNA replication and repair, transcription regulation and genome 3D organization. Cohesin is often mutated (up to 20%) in solid and hematologic cancers. It is thus essential to decipher the molecular basis of Cohesin mechanisms to understand how its mutations lead to cancers.

Cohesin is composed of 4 core proteins: SMC1A, SMC3, RAD21 and STAG1/2, which adopt a ring-shaped structure allowing the DNA entrapment. SMC1A and SMC3 are constituted of an ATPase head connected to a hinge domain. They heterodimerize through their hinge domains. Interaction of the N- and C-terminal regions of RAD21 with SMC3 and SMC1A ATPase heads closes the Cohesin ring, STAG1/2 interacting with the RAD21 central region.

Methods

The ATPase activity is essential to the multiple Cohesin functions. ATP binding causes the heterodimerization of SMC1A and SMC3 ATPase heads and ATP hydrolysis their dissociation. Numerous cancer-associated mutations are located in the ATPase module, formed by SMC1A and SMC3 ATPase heads, RAD21 and STAG1/2, but also in Cohesin regulatory proteins interacting with this module. My project aims at characterizing the dynamics of the ATPase module formation upon ATP binding and how cancer-related mutations perturb its dynamics.

Results

I have initially focused my work on the SMC1A and SMC3 ATPase heads engagement in complex with RAD21 and ATP. My biochemical and biophysical results have revealed the dynamics of this engagement, notably the extreme sensibility of this interaction to slight changes. These findings suggest that even small perturbations caused by cancer-related mutations may have a major effect on Cohesin functions. My structural analyses of engaged ATPase heads by cryo-electron microscopy also support and refine my initial conclusions, highlighting the stable and dynamic regions of this module.

Conclusions & perspectives

My present work consists of adding the STAG1/2 subunit to this initial complex to analyze how and if it may affect the dynamics of the ATPase module. I am currently analyzing the effect of cancer-induced mutations on these dynamics. My new results will allow a deeper understanding of the human Cohesin ATPase module dynamics both in physiological and pathological conditions.

PRIX HÉLÈNE STARCK 2020

LOTZ CHRISTOPHE // Doctorant
Responsable scientifique : LAMOUR Valérie
Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch-Graffenstaden

Etudes fonctionnelles et structurales des ADN topoisomérases de type II et de leurs modifications post-traductionnelles

Context & Objectives

Type II DNA topoisomerases (TopIIs) are proteins involved in many cellular processes. They regulate DNA topology by cutting DNA. Based on this mechanism, therapeutics inhibit the catalytic activity of these enzymes. Administration of those compounds to patients can trigger side effects, urging researchers to develop new molecules. Structural information on the possible conformations that TopIIs can adopt during the catalytic cycle and regulation by post-translational modifications (PTMs) are necessary to design new therapeutic compounds.

Results

The structure of whole nucleoprotein complexes of prokaryotic and eukaryotic TopIIs with therapeutic molecules was determined by cryo-electron microscopy.These structures revealed prokaryotic/eukaryotic structural differences, particularly in the connections between domains. The study of the catalytic activities of enzymes after directed mutagenesis showed that these connections are important for allosteric communicationsbetween domains and determined by species-specific motifs.

We have studied the regulation of TopII by PTMs. The identification of phosphorylation and acetylation sites revealed the role of a conserved lysine in the ATP binding domain of the TopII, subject to acetylation. This lysine is important for the coupling between ATP hydrolysis and dimerization of the N-terminal domain. PTMs can affect conserved positions, affecting the structure-function relationship of the protein which regulate its activity during the catalytic cycle.

A review of the literature allowed us to compare the PTMs identified in cancer cells with the PTMs identified in normal cells. TopII in cancer cells have different distribution of PTMs, indicating that PTMs might be related to the status of the cells and could potentially predict their response to treatments targeting the TopII.

Conclusion & Perspectives

Although prokaryotic and eukaryotic enzymes possess catalytic domains with strong sequence similarity, their activities are finely regulated by conserved motifs located in the connections between these domains. In addition, PTMs in TopII bring an additional degree of complexity and have an impact on the structure-function relationship and allosteric control of the TopII activities. PTMs identified in the literature on TopII from cancer cells can vary according to cell type and these differences could be explored to study the interplay between PTMs and the resistance to anti-cancer treatment.

Publications related to the funded project

Vanden Broeck, A et al. *"Structural basis for the allosteric regulation of Human Topoisomerase 2a "* submitted

Lotz, C;Lamour, V. *"The interplay between DNA topoisomerase 2a post-translational modifications and drug resistance"*

Vanden Broeck, A et al.*"Cryo-EM structure of the complete E. coli DNA Gyrase nucleoprotein complex"*

Bedez, C;Lotz, C et al. *"Post-translational modifications in DNA topoisomerase 2a highlight the role of a eukaryote-specific residue in the ATPase domain."*

NICOLAS-BOLUDA ALBA // Doctorante
Responsable scientifique : GAZEAU Florence
Institut Cochin, équipe Matieres et Systemes Complexes, Paris

Targeting lysyl oxidase (LOX) favors T cell migration in the tumor stroma and enhances anti-PD1 treatment.

Cancer immunotherapy is a promising therapeutic intervention. However, complete and durable responses are only seen in a fraction of cancer patients. One of the determinants in the success of T cell-based immunotherapies lies in the ability of effector T cells to reach tumor cells. Solid tumors are characterized by an aberrant organization of the extracellular matrix (ECM) in the form of highly reticulated and long linear collagen fibers, which have been shown to limit T cells infiltration into tumor cell islets. Currently, there are several strategies in development to target the tumor ECM including the inhibition of lysyl oxidase, an extracellular copper-dependent enzyme upregulated in many tumors that catalyzes the cross-linking of collagen. Here, using several relevant preclinical mouse models of pancreatic, breast and bile duct carcinomas combined with dynamic imaging on fresh tumor slices, we investigated the consequences of LOX pharmacological inhibition with beta- aminopropionitrile (BAPN) on several parameters (tumor mechanical properties, intratumoral migration of T cells, tumor growth). Our data indicate that treatment of mice with BAPN leads to a significant decrease of tumor stiffness mapped using shear wave elastography that correlates with an increase of T cell intratumoral migration. Although this treatment alone has minor effects on tumor growth, its combination with anti–PD-1 therapy increases the accumulation of effector CD8 T cells and delays tumor progression. This study highlights the rationale of combining approaches targeting the ECM and immune checkpoint proteins.

PRIX HÉLÈNE STARCK 2020

ROSINSKA SARA // Post-doctorante
Responsable scientifique : GAVARD Julie
Centre de Recherche en Cancérologie et Immunologie Nantes Angers, équipe SOAP

Involvement of Junctional Adhesion Molecules C (JAM-C) in Communication between Glioblastoma Stem-like Cells and Brain Endothelial Cells

Context & Objectives

Glioblastoma (GBM) is the most aggressive and common malignant primary brain tumor in adults with a high recurrence and mortality rate. The presence of Glioblastoma Stem-like Cells (GSCs) are suspected to be responsible for tumor initiation, expansion, recurrence and resistance to current therapies. Moreover, GSCs localize in close proximity to brain blood vessels which supports not only their growth, but also allows GSCs to migrate along the vasculature and to invade healthy tissues. GSCs are highly migratory and the invasive nature of GBM is related to the intracranial spreading of GSCs using tumor vasculature. The adhesion molecules engaged in direct tethering of GSCs to the brain endothelial cells (EC) that lines blood vessels, are not identified so far as well as the mechanism of invasion.

Methods

Our transcriptomic analysis identified Junctional Adhesion Molecules C (JAM-C) as a putative adhesion partner between GSCs and ECs. In silico analysis confirmed higher JAM-C RNA expression in GBM than in non-tumoral brain tissue, moreover, increased JAM-C expression correlates with poor probability of survival of GBM patients. We therefore investigated the impact of JAM-C on the tumor vascular niche, by developing ex vivo human models. First, RNA silencing (knock-down, KD) or gene deletion (knock-out, KO) of JAM-C in GSCs hampers adhesion to EC monolayers. Moreover, JAM-C KO reduced the levels of SOX2 and NESTIN stemness markers. This was accompanied by an elevated differentiation ability of GSCs, in terms of morphological and molecular differentiation, adhesion and sprouting migratory behavior from 3D spheroids.

Results

Our results established that JAM-C acts at the level of both GSC/GSC and GSC/EC interaction, impacting their binding and migratory behavior and the stem-like properties of GSCs. Future studies will decipher whether JAM-C disruption benefits to tumor expansion and/or invasion either in vitro as in vivo.

VUGIC DOMAGOJ // Doctorant
Responsable scientifique : CARREIRA Aura
Institut Curie, UMR3348, Orsay

Interplay between the two DNA binding domains of BRCA2 protein

Germline mutations in the BRCA2 gene predispose to increased risk of breast and ovarian cancer. BRCA2 is one of the key mediators of Homologous recombination (HR) and is also involved in protecting stalled replication forks from excessive nucleolytic degradation by MRE11. BRCA2 contains a DNA binding domain at the C-terminus (CTD) and recently reported a novel DNA binding site in the N-terminus (NTD) that can promote HR in vitro. Since BRCA2 has two DNA binding domains, the canonical one at the C-terminus (CTD) and a novel one at its N-terminal part (NTD), we hypothesized that these two DNA binding domains of BRCA2 may have evolved to repair specific types of DNA damage. We generated stable cell lines expressing variants identified in breast cancer patients altering the DNA binding activity of the CTD or the NTD. We then characterized their phenotype in the context of DSB repair and/or replication stress. We demonstrate that, in contrast to the CTD variant bearing cells, cells expressing the NTD variants are HR proficient and are not sensitive to PARP inhibitor treatment. Interestingly, we observe an increased sensitivity of NTD variants upon Hydroxyurea (HU). Moreover, we show that cells expressing BRCA2 NTD variants display a defect in localization of both BRCA2 and RAD51 to the nascent DNA compared to the WT BRCA2. Our data suggest that BRCA2 CTD is required for the HR (Double-strand break repair) and that the NTD could be involved in the protective function of BRCA2 at stalled replication forks. The same experiments performed with a thymidine chase revealed the specific recruitment of BRCA2 to the nascent DNA, whereas RAD51 appear bound to chromatin. Furthermore, our results point to replication fork not being restrained for the BRCA2 deficient cells and BRCA2 C315S variant upon treatment with Hydroxyurea that is not the case for CTD R3052W variant nor S3291A mutant. This leads to accumulation of single stranded gaps that are know to be marker of genomic instability and factor of resistance to chemotherapy.

JJC 2020

PRIX KERNER

MEMBRES DU JURY

BRIGITTE BLOND, Santé Magazine, Femme Actuelle, Ouest France
CHARLINE DELAFONTAINE, Pleine Vie
KARELLE GOUTORBE, Le Quotidien du Médecin
ISABELLE GRILLOT, Science & Vie TV
SUZY JOURDAN, Côté Santé
SANDRINE MOUCHET, Rose Magazine
ALINE PERRAUDIN, Santé Magazine
FABIENNE RIGAL, Femme Actuelle
AFSANÉ SABOUHI, Ça m'interesse
JULIE WIERZBICKI, Pharmaceutiques

1 AUDOYNAUD CHARLOTTE // Doctorante

Institut Curie, Orsay

Cancérogenèse : comment se protegent les cellules ?

Le processus cancérogène est contrebalancé par des mécanismes de défense efficaces des cellules du corps. Charlotte Audoynaud, doctorante à l'Institut Curie, nous fait part de l'intérêt de ses travaux sur la compréhension de ces mécanismes de protection, un domaine au cœur de la recherche fondamentale en cancérologie actuelle.

2 FARGE THOMAS // Doctorant

Centre de Recherche en Cancérologie de Toulouse

Quand les cellules leucémiques s'évadent pour résister

Dans une équipe du Centre de Recherche en Cancérologie de Toulouse, un projet de thèse, soutenu par la Fondation ARC, part à la recherche des cellules leucémiques les plus résistantes aux traitements. Cette étude se penche sur un mécanisme de résistance qui, jusqu'ici, avait été ignoré : les cellules leucémiques sont capables de coloniser de nouveaux organes afin d'éviter l'action de la chimiothérapie.

3 GREGORY JULES // Doctorant

Centre de Recherche en Epidémiologie et Statistiques, Paris

Recherche sur la chimioembolisation intra-artérielle pour le traitement du carcinome hépatocellulaire : une quantité choquante de résultats non disponibles.

La radiologie interventionnelle (RI) est une discipline jeune, extrêmement innovante et qui a pris une place importante au sein des traitements contre le cancer. Évaluer l'impact d'innovations rapides et techniques en santé n'est pas aisé mais nécessaire au vu des investissements financiers et du fardeau que peut représenter la recherche pour les patients. Aussi, l'objectif de ce doctorat est, dans un premier temps, d'évaluer les problématiques auxquelles la recherche en RI est confrontée, afin de proposer des outils méthodologiques d'évaluation thérapeutique adaptés. Dans un second temps, d'évaluer ces outils sur une série d'innovations thérapeutiques en cours. Pour ce faire nous avons choisi comme modèle d'étude la chimio-embolisation intra-artérielle (CEA) du carcinome hépatocellulaire (CHC), traitement ayant donné lieu à une recherche conséquente contre un cancer fréquent avec une mortalité élevée.

4 LICAJ MONICA // Doctorante

Institut Curie, Paris

Cancer de l'ovaire : On pense souvent aux cellules tumorales mais on oublie le reste ! Vers un nouveau traitement et au delà.

Chaque année en France, 4500 femmes sont diagnostiquées du cancer de l'ovaire. En raison d'un diagnostic souvent tardif, le pronostic reste sombre avec un taux de survie globale à 5 ans de 45%. Les traitements d'aujourd'hui ne sont pas suffisants pour guérir le cancer de l'ovaire chez les femmes. Mes recherches ont permis d'ouvrir la voie vers un traitement complémentaire à la chimiothérapie, plus efficace. Il s'agit d'éliminer les cellules de soutien appelées vulgairement CAF-S1 en ciblant YAP1, un élément qui joue un rôle clef dans la chimiorésistance.

5 LIGIER MAUD // Doctorante

Centre de Recherche en Cancérologie de Lyon

Mélanome, le tueur qui échappe à nos défenses. Comment fait-il et comment l'arrêter ? ZEB1 une piste sérieuse

Le mélanome malin est une forme très agressive de cancer de la peau. Si la chirurgie, réalisée au stade précoce, est très efficace, la prise en charge des patients atteints de mélanome avancé est bien complexe. Deux stratégies existent : les thérapies ciblées qui ciblent une spécificité génétique de la tumeur et les immunothérapies qui visent à activer le système immunitaire propre du patient à lutter contre la tumeur. Ces traitements sont des avancées majeures dans le traitement des mélanomes. Cependant, plus de la moitié des patients ne répondent pas à ces thérapies ou rechutent, car le mélanome a une capacité incroyable à s'adapter à son environnement changeant afin de résister aux thérapies. Les travaux de Maud Ligier, doctorante au Centre de recherche en cancérologie de Lyon sont centrés sur ce phénomène d'adaptation de la tumeur, cherchant à comprendre les mécanismes de résistance aux traitements dans le but de les prévenir et/ou de les contrer.

6 MORETTI CHARLOTTE // Post-doctorante

Institut de Génomique Fonctionelle de Lyon

Comment les gènes sont-ils contrôlés dans nos cellules ?

Chacune de nos cellules renferment exactement la même information génétique. Et pourtant, toutes nos cellules ne se ressemblent pas ! A l'origine de cette diversité ? Des centaines de milliers de séquences contenues dans notre ADN qui sont capables de contrôler l'activité de nos gènes. Focus sur les travaux de recherche d'une équipe de l'Institut de Génomique Fonctionnelle de Lyon qui étudie comment ces séquences mènent nos gènes à la baguette...

7 PEGLION FLORENT // Post-doctorant

Institut Pasteur, Paris

Tumeurs Cérébrales : les nageoires de l'espoir

Dans un laboratoire de l'Institut Pasteur, des petits poissons aux rayures blanches et noires aident les chercheurs à comprendre comment les tumeurs du cerveau se propagent. En transplantant des cellules cancéreuses de patients dans les larves transparentes du poisson-zèbre, le Dr Florent Peglion suit en temps réel la façon dont elles s'infiltrent dans le cerveau. Ses travaux doivent permettre de développer de nouveaux traitements bloquant la progression fatale de ces tumeurs.

8 THYS AN // Post-doctorante

Université de Nantes

Modification de SHARPIN : un pacte avec le diable?

La Dr An Thys, postdoctorante Belge dans le laboratoire SOAP au sein du CRCINA, parle de ses découvertes en recherche fondamentale sur SHARPIN, une protéine importante dans l'activation des globules blancs et des lymphomes.

LISTE DES CANDIDATS

CATÉGORIE POST-DOCTORAT

58 ABOUELMAATY Mohamed, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch
28 AL JORD Adel, Collège de France, Paris
66 BASBOUS Sara, Université de Bordeaux
20 BRUNEL Benjamin, Université de Reims Champagne-Ardenne
31 FABRE Bertrand, Laboratoire de Recherche en Sciences Végétales (LRSV), Auzeville-Tolosane
21 GORVEL Laurent, Centre de Recherche en Cancérologie de Marseille
67 GOUNGOUNGA Juste, Université de Bourgogne, Dijon
40 GUILLORY Xavier, Université de Rennes 1
22 KRAMARZ Karol, Institute Curie, Orsay
23 MOINDJIE Hadia, Gustave Roussy, Villejuif
75 MORETTI Charlotte, Institut de Génomique Fonctionnelle de Lyon
24/75 PEGLION Florent, Institut Pasteur, Paris
52 POTY Sophie, Institut de Recherche en Cancérologie de Montpellier
71 ROSINSKA Sara, Centre de Recherche en Cancérologie et Immunologie Nantes Angers
25 SMITH Rebecca, Institut de Génétique et Développement de Rennes
45 SUAREZ Guadalupe, Institut Curie, Paris
75 THYS An, Université de Nantes
34 TRAN Vu Long, Service Hospitalier Frédéric Joliot, Orsay
54 Van BODEGRAVEN Emma, Institut Pasteur, Paris
35 VENE Élise, Institut NuMeCan, Rennes
55 VOISIN Allison, Centre de Recherche en Cancérologie de Lyon
64 ZHOU Ya, Institut Curie, Paris

CATÉGORIE THÈSE

74 AUDOYNAUD Charlotte, Institut Curie, Orsay
59 BEKKAT Fériel, Institut Cochin, Paris
10 CASANOVA Alexandre, Institut pour l'Avancée des Biosciences, La Tronche
11 CLUZET Victoria, Université Paris Diderot
30 DUFRESNE Suzanne, Université Rennes
38 DUMETIER Baptiste, Université de Bourgogne, Dijon
12/74 FARGE Thomas, Centre de Recherche en Cancérologie de Toulouse
48 FOURNIE Claire, Université de la Réunion, Le Tampon
13 GHOROGHI Shima, Université de Strasbourg
39 GIACCHERINI Cédric, Centre de Recherche en Cancérologie de Marseille
49 GIMENEZ ANDRES Manuel, Institut Jacques Monod, Paris
74 GREGORY Jules, Centre de Recherche en Epidémiologie et Statistiques, Paris
14 HERVÉ Solène, Institut Curie, Paris
15 JACOBS Kathryn, Centre de Recherche en Cancérologie et Immunologie de Nantes Angers
16 JULIEN Manon, Institut de Biologie Intégrative de la Cellule, Gif-sur-Yvette

LISTE DES CANDIDATS

68 LANDWERLIN Pauline, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch
32 LEBDY Rana, Institut de Génétique Humaine, Montpellier
41/74 LICAJ Monika, Institut Curie, Paris
17/75 LIGIER Maud, Centre de Recherche en Cancérologie de Lyon
60 LIU Jing, Institut Curie, Paris
69 LOTZ Christophe, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch
51 LOZANO Anthony, Institut de Génétique Moléculaire de Montpellier
43 MENNOUR Sabrina, Institut Curie, Orsay
53 MIRO PINA Caridad, Institut Jacques Monod, Paris
70 NICOLAS-BOLUDA Alba, Institut Cochin, Paris
33 PETRAZZUOLO Adriana, Centre de Recherche des Cordeliers, Paris
62 PINTO Giulia, Institut Pasteur, Paris
44 ROUSSEAU Mélanie, Centre de Recherche en Cancérologie de Montpellier
63 VIAL Anthony, Centre de biochimie structurale, Montpellier
72 VUGIC Domagoj, Institut Curie, Paris

CATÉGORIE MASTER

29 ALRIC Hadrien, Paris Centre de Recherche Cardiovasculaire
50 HAMANN Pierre, Gustave Roussy, Villejuif
42 MAINGUENÉ Juliette, Institut Curie, Paris
61 PARENT Pauline, Institut Pasteur de Lille

LISTE DES CANDIDATS

La Fondation ARC pour la recherche sur le cancer

Reconnue d'utilité publique, la Fondation ARC est 100 % dédiée à la recherche sur le cancer. Grâce à la générosité de ses donateurs et testateurs, elle alloue chaque année plus de 26 millions d'euros à des projets de recherche porteurs d'espoir pour les malades. Son objectif : contribuer à guérir 2 cancers sur 3 en 2025.

La Fondation ARC a pour mission de lutter contre le cancer par la recherche. Forte d'une expertise nationale et internationale de très haut niveau, elle met en œuvre une action scientifique déterminée visant à accroitre les connaissances sur tous les cancers, à favoriser l'innovation thérapeutique et à créer les conditions d'une recherche d'excellence.

Menée en toute indépendance et sur l'ensemble du territoire, son action est guidée par l'intérêt général et l'excellence scientifique : elle identifie, sélectionne, finance et accompagne des programmes de recherche prometteurs. La Fondation ARC est un véritable catalyseur de la recherche et fédère ainsi les acteurs de la lutte contre le cancer en France et à l'international.

La Fondation ARC est exclusivement financée par la générosité du public. Elle est agréée par l'organisme de contrôle le « Don en confiance » depuis 1999.

DON en CONFIANCE comitecharte.org

FONDATION ARC POUR LA RECHERCHE SUR LE CANCER

Reconnue d'utilité publique

9, rue Guy Môquet - BP 90003 - 94803 Villejuif Cedex - Tél : 01 45 59 59 59