JEDNs 85

Journées de l'école doctorale de Nice

KEYNOTE SPEAKERS
Emmanuel Barillot
Halyna Shcherbata
Stefan Stefanovic

27 - 28 may 2019
Chemistry Amphitheatre
Valrose Campus, Nice, France

Abstract Book

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ÉCOLE DOCTORALE 85
Sciences de la Vie et de la Santé

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Keynote lecture

This year the JEDNs are pleased to welcome:

Emmanuel Barillot is a researcher who is also **head of U900 at Curie Institute**. With his team, he uses bioinformatic approaches to study molecular and cellular aspects of cancer (initiation, development, and progression of tumors as well as enhancing therapeutic strategies)

Halyna Shcherbata is an EMBO Young investigator**, leading her group at the Max Planck Institute for Biophysical Chemistry in Gottingen**, Germany. Her scientific interests are ranging from understanding the role of miRNAs in regulating stem cells and cellular differentiation in normal and stress condition to the study of muscular dystrophy underlying mechanisms using Drosophila as a model organism.

Stefan Stefanović is a **Professor of Molecular Immunology at the University of Tübingen**. His work is focused on HLA molecule understanding through motif determination, ligandome mapping, and screening of T cells reactions against viral epitopes.

Scientific committee

Many thanks to the jury members that volunteered:

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Dries Amezian (1st yr, ISA), Raphaël Bonnet (1st yr, C3M), Nadia Formicola (4th yr, IBV)
Sol Jacome (2nd yr, IRCAN), Alphy John (2nd yr, IBV), Baptiste Ropert (2nd yr, IRCAN),
Vivien Weber (3rd yr, IRCAN)

Detailed Program

Monday May 27th

8h00 – 8h45 Registration/breakfast

8h45 – 9h00 Opening and welcoming talk by Dr Thomas Lamonerie, Director of the doctoral school

9h00 – 10h00 Invited Keynote Lecture: **Stefan Stefanović**: Department of Immunology, University of Tübingen – Germany

10h00 – 11h00 Oral Communication Session 1

OC-1 Serena Diazzi
The pro-fibrotic miR-143/145 cluster regulates an extracellular matrix (ECM) remodeling program during adaptive and acquired resistance of melanoma cells to targeted therapies.

OC-2 Fabien Muselli
New pharmacological approaches to target persistent Leukemic Stem Cells in Chronic Myeloid Leukemia.

OC-3 Marwa Zerhouni
Targeting metabolism through covalent inhibition of PKM2 in cancer treatment.

OC-4 Bérengère Dadone-Montaudié
The FGF/FGFR signaling pathway: a novel therapeutic target in liposarcomas.

OC-5 Mickael Meyer
Studying the single-cell response heterogeneity of tumor cells to death ligand drugs.

OC-6 Henri Montaudié
CLEC a new actor in melanoma

11h00 – 11h20 Coffee Break

11h20 – 12h20 Oral Communication Session 2

OC-7 Rana Mhaidly
GAPDH overexpression in the T cell lineage in vivo promotes a T cell lymphoma

OC-8 Sofia Fazio
Exploring the function of Ro60 complex in lipid-laden macrophages

OC-9 Giorgia Miloro
Revisiting the regulation of T cell activation by Fas receptor: critical involvement of the SHP-1 phosphatase.

OC-10 Charlène Iltis
Senescent cells bypass NK cell mediated immunosurveillance through a GD3 ganglioside dependent pathway.

OC-11 **Maria-Bernadette Madel**
Heterogeneity in inflammatory osteoclasts provokes alterations in their inflammatory phenotype and bone resorption capacity

OC-12 **Iris Grosjean**
Human Endogenous RetroVirus and Immune evasion in lung cancer.

12h20 – 13h10 Lunch Break

13h10 – 14h30 Poster session 1 (from **P-1** to **P-18**)

14h30 – 15h30 Invited Keynote Lecture: **Emmanuel Barillot**: Team Leader, Institut Curie – France

15h30 – 16h30 Oral Communication Session 3

OC-13 **Lucilla Fabbri**
Identification of a new pro-death axis in ccRCC patients driven by ciliogenesis and absence of VDAC1-ΔC.

OC-14 **Charlotte Pandiani**
Identification and characterization of intratumoral heterogeneity in uveal melanoma.

OC-15 **Christopher Rovera**
Factors secreted by invasive melanoma cells inhibit the contraction of lymph node fibroblasts.

OC-16 **Rouba Jneid**
Btk toxins influence progenitor cell fate of intestinal stem cells.

OC-17 **Montserrat Vásquez Rojas**
The role of EFA6B in epithelial morphogenesis and its implication in breast cancer progression

OC-18 **Marine Gautier**
Functional characterization of hypoxia-regulated lncRNAs in Non-Small Cell Lung Carcinomas.

16h30 – 16h50 Coffee Break

16h50 – 17h50 Oral communication Session 4

OC-19 **Serena Silvano**
Deciphering the role of Z1 and Z3 in the pancreas.

OC-20 **Pierre Leclère**
Role of the circadian transcription factor Krüppel like factor 10 in Nonalcoholic Steatohepatitis (NASH).

OC-21 **Ahmed Negm**
Lipid rich diet induce inflammation and thermal pain through ASIC3 activation.

OC-22 **Joffrey Mejias**
A root-knot nematode effector targets the spliceosomal plant machinery facilitating the giant cells formation.

OC-23 **Marie Deprez**
Single-cell RNA sequencing reveals novel cell differentiation dynamics during human airway epithelium regeneration.

OC-24 **Jocelyn Gal**
Comparison of unsupervised machine learning methods in localised breast cancer patients based on metabolomics signatures.

17h50 – 19h00 Drinks

19h00 – 21h30 Dinner

Tuesday, May 28th

8h30 – 9h Registration

9h00 – 10h00 Invited Keynote Lecture: **Halyna Shcherbata**: Team Leader, Max Planck Institute – Germany

10h00 – 11h00 Oral Communication Session 5

OC-25 **Kavya Vinayan Pushpalatha**
Age-dependent remodeling of neuronal RNP granules.

OC-26 **Marta Prieto**
Impact of a Fragile X Syndrome missense mutation on the neuronal function.

OC-27 **Małgorzata Drozd**
Functional characterization of new genes implicated in Early Onset Schizophrenia and Autism.

OC-28 **Maria Mensch**
A-eta-alpha: another app protein with impact on hippocampal synaptic function or why it is important to have an overview before we try to cure.

OC-29 **Sandra Dhifallah**
Negative dominance: a novel mechanism in SCN2A mutations causing Autism.

OC-30 **Tsai Meng-Chen**
Polyunsaturated phospholipid incorporation in host membranes attenuate bacterial invasion.

11h00 – 11h20 Coffee Break

11h20 – 12h20 Oral Communication Session 6

OC-31 **Audrey Valverde**

Contributions of aminopeptidase A and dipeptidyl peptidase IV to N-terminal truncations of Aß peptide : in vitro, ex vivo and in vivo approaches.

OC-32 Pablo Ávalos Prado
KCNE1 activates the voltage-dependent and calcium-activated chloride channel TMEM16A.

OC-33 Pensieri Pasquale
Role of transcription factor Otx2 in adult retina photoreceptors.

OC-34 Chiara Tocco
Morphological and electrophysiological characterization of layer V subcortical projection neurons in a transgenic mouse model of fine motor skill impairments.

OC-35 Lamya Khoubza
Conserved gating of vertebrate and invertebrate two-pore K+ channels.

OC-36 Tatiana Gritsaenko
Characterization of bone extracellular matrix produced by recql4-deficient osteoblaststle.

12h40 – 13h10 Lunch Break

13h10 – 14h30 Poster Session 2 (from **P-19** to **P-35**)

14h30 – 15h30 Oral communication Session 7

OC-37 Camille Syska
VapBC toxin-antitoxin modules of Sinorhizobium meliloti: actors of the nitrogen-fixing symbiosis.

OC-38 Laïla Giordano
The Arabidopsis Receptor-Like Kinase IOS1 links filamentous pathogen attack to Endoplasmic Reticulum stress.

OC-39 Gaurav Pandharikar
Cross-talk between aphid facultative symbionts and nitrogen fixation symbiosis in the aphid and legume interaction.

OC-40 Li Yang
Regulation of senescence-specific proteases during the nitrogen-fixing symbiosis in Medicago truncatula.

OC-41 Thomine Eva
Increasing crop diversity in space and time at a filed scale to promote sustainability of agricultural systems.

OC-42 Cristina Paraschivescu
The role of CCL17 in the murine brain development and behaviour.

15h30 – 15h50 Coffee Break

15h50 – 16h50 Oral Communication Session 8

OC-43 Alice Rouan
Environmental and genetic impact on telomere size dynamic in 3 coral species across the Pacific Ocean.

OC-44 Morgane Plutino
Defect of mitochondrial pseudouridylation : Identification of a novel mitochondrial disease gene ?

OC-45 Charles Puerner
Mechanical forces during filamentous growth of a human fungal pathogen.

OC-46 Paula Peressini
LINE-1 retrotransposition in murine and human muscle cells.

OC-47 Melania D'Angiolo
Chronic telomeric stress impairs global organismal fitness and genomic stability in budding yeast.

OC-48 Simone Mozzachiodi
Overcoming genome complexity barriers in hybrids with meiotic reversion.

16h50 – 17h10 Coffee Break

17h10 – 18h10 Oral Communication Session 9

OC-49 Anaïs Bécot
Altered gamma-secretase function leads to the enrichment of high molecular weight APP-CTFs in brain exosomes from Alzheimer mouse models.

OC-50 Loïc Brussot
The laterodorsal tegmental nucleus: a new actor in freezing.

OC-51 Julien Marcetteau
The role of Arf6 in Wg/Wnt signalling.

OC-52 Bénédicte Billard
Evolution of developmental plasticity in the nematode C. elegans.

OC-53 Marion Tiberti
An optimal distribution of polyunsaturated acyl chains in phospholipids for fast membrane deformation and fission by endocytic proteins.

OC-54 Romain Rozier
Protective mechanisms of pharmacologic preconditioning against myocardial ischemia reperfusion injury: role of Bcl-2 family proteins.

18h10 Closing session and JEDNs award

Poster Index

Session 1 (Monday, May 27th)

P-1 **Adriana MARTINEZ-TURTOS**
Low protein diet enhances tumor immunogenicity through activation of IRE1α

P-2 **Elisa CAVAZZA**
Potentiation of immune checkpoint inhibitors by new anti-melanoma compounds

P-3 **Elodie VIEIRA**
CD44 regulates NKp46+ Innate Lymphoid Cells behavior and their cross-talk with inflammatory macrophages during non alcoholic steatohepatitis

P-4 **Johanna MERLIN**
Myeloid cell glutaminolysis controls monocyte numbers and macrophage efferocytosis during atherosclerosis

P-5 **Manuel GRIMA**
Understanding asparagine synthetase heterogeneity and its impact in the metabolism of B-cell lymphomas

P-6 **Margaux LECACHEUR**
Role of mechanotransduction in melanoma cell plasticity and progression

P-7 **Marion STUNAULT**
Adipose tissue derived fatty acids control monocyte mobilization

P-8 **Sébastien LE GARF**
Exercise and PPARβ agonist treatment improve immunometabolic and aerobic capacities in the context of diet-induced weight loss in obese female mice

P-9 **Anna GARRIDO-UTRILLA**
Turning intestinal somatostatin+ cells into beta-like cells

P-10 **Marika Elsa FRIANO**
The role of the transcription factor E2F1 in pancreatic alpha/beta-cell identity and plasticity.

P-11 **Aurore DUMOND**
Opposite effects of Neuropilin-1 and Neuropilin-2 in the aggressiveness of clear cell Renal Cell Carcinoma

P-12 **Miguel-Angel BASANTE-BEDOYA**
The phosphatidylserine flippase Drs2 has a unique role during Candida albicans invasive growth

P-13 Siyue DU
Impact of Vascular PPARbeta/delta Expression on Tumor Progression and Metastasis Formation

P-14 Ophélie VERMEULEN
Newly infiltrated neutrophils under CpG + aIL10Ra peritumoral treatment play a key role in antitumor immunity and tumor regression

P-15 Boutaina DAHER
Genetic disruption of the cystine importer xCT (SLC7A11) reduces growth, survival and tumorigenicity and increases sensitivity to chemotherapy of pdac cells

P-16 Jonathan BENZAQUEN
Non-small cell lung cancer infiltrating immune cells express a truncated P2RX7 splice variant impairing the proper localization of P2RX7 to the cell membrane

P-17 Radia Zeghari
Multimodal and Multidimensional assessment of apathy

P-18 Djampa KOZLOWSKI
Transposable Elements activity and role in Meloidogyne incognita genome dynamic and adaptability.

Session 2 (Tuesday, May 28th)

P-19 Alphy JOHN
A two-tier junctional machinery under the control of embryo cross-patterning drives coordinated tissue folding and elongation

P-20 Ioannis OIKONOMAKOS
Organoids of intermediate mesoderm derived tissues from mouse ES cells

P-21 Leandro CORREA
Identification of active module in an interaction graph using node2vec network embedding

P-22 Malalaniaina RAKOTOBE
Effects of the interaction between chronic stress and Otx2 expression on the habenula-interpeduncular functions

P-23 Arnaud MARY
Role of AMPK in mitochondrial dysfunctions and neuroinflammation in Alzheimer disease

P-24 Bonnie LABRUM
Lysophosphatidylcholine modulates ASIC channels and induces long-lasting joint pain in mice in an ASIC3-dependant manner

P-25 Marie PRONOT
Characterization of a synaptic SUMO2/3-ylome.

P-26 Méliné SIMSIR
On the export mechanism by RND proteins: AcrB's structure-based study

P-27 Nicolas-Frédéric LIPP
An electrostatic switching mechanism controls the activity of PS / PI4P exchanger

P-28 Martin BERMUDEZ
Gut microbial metabolite contributes to the development of autistic-like behaviours in mice

P-29 Sébastien DELHAYE
Targeting Phosphodiesterase 2A in the Fragile X Syndrome

P-30 Baptiste ROPERT
Therapeutic strategies for mitochondrial diseases using cellular and murine model.

P-31 Lou DURET
Study of the regulation of telomerase activities by a phosphatase called pX.

P-32 Martin REY-MILLET
Role of the telomeric protein TRF2 in post-mitotic cells.

P-33 Olivia VIDAL CRUCHEZ
XRN1: a protein at the crossroad between pbodies and cytoplasm

P-34 Maria Sol JACOME-BURBANO
FOXO3a role for telomeres homeostasis and skeletal muscle aging.

P-35 Flora AUBREE
The temporal varibility of migration can decrease the expected genetic differencition of sub-divided populations

Oral Communication Abstracts

Session 1

OC-1

The pro-fibrotic miR-143/145 cluster regulates an extracellular matrix (ECM) remodeling program during adaptive and acquired resistance of melanoma cells to targeted therapies

Diazzi S. (1,2), Vassaux G. (2), Girard C. (1), Fassy J. (2), Lecacheur M. (1), Berestjuk I. (1), Deckert M. (1), Mari B.* (2), Tartare-Deckert S.* (1)

1.Inserm, U1065, Team Microenvironment, Signaling and Cancer, C3M, Université Côte d'Azur, Nice, France. Équipe labellisée Ligue Contre le Cancer 2016; 2. Institut de Pharmacologie Moléculaire et Cellulaire, UMR 7275 CNRS and Université Côte d'Azur, Valbonne, France.

Melanoma cells are well known for their plasticity and ability to phenotype switch toward an invasive de-differentiated mesenchymal state(1). The emergence of this mesenchymal phenotype has been shown during primary, adaptive and acquired resistance to MAPK-targeted therapies in BRAF mutant melanoma cells (2). The mesenchymal acquired resistance is achieved by overexpression of receptor tyrosine kinases (3) and we evidenced that this cell state is associated with expression of markers observed in fibrotic diseases and acquisition of cancer-associated fibroblasts (CAFs)-like ECM remodeling activities to generate a drug-tolerant microenvironment.

However, epigenetic or post-transcriptional signaling networks that regulate this phenotype remain to be defined. In this perspective, our study focuses on the characterization of a pool of microRNAs (miRNAs) involved in fibrotic diseases, called "FibromiRs" (4).

Here, we identify the pro-fibrotic miR-143/145 cluster as a good candidate for the ECM program activation in drug-tolerant melanoma cells through the regulation of ECM deposition and remodeling and its contribution in acquisition of invasiveness and mesenchymal traits. The two miRNAs are found strongly overexpressed in mesenchymal resistant cells versus sensitive cells and induced upon TGF-β or PDGF stimulation as well as in response to MAPK signaling pathway inhibition in mutant BRAF V600E melanoma cell lines.

In addition, inhibition of TGF-β or PDGF signaling pathways in drug resistant cells leads to a decrease in cluster expression, meaning that constitutive activation of these pathways, typical of mesenchymal resistant state, might be responsible for activation of pro-fibrotic miR-143~145 cluster expression.

Overall, our data indicate that the miR-143/145 cluster contributes to cell plasticity and acquisition of CAFs-like ECM remodeling activities that occur during the rewiring of melanoma cells signaling in response to MAPK-targeting therapies.

1. Hoek et al., Cancer Research, 2008 2. Arozarena et al., Annals of Translational Medicine, 2017 3. Nazarian et al., Nature, 2010 4. Pottier et al., Trends in Pharmacological Sciences, 2014

OC-2

New pharmacological approaches to target persistent Leukemic Stem Cells in Chronic Myeloid Leukemia

Fabien MUSELLI1, Anne-Laure ROSSI1, Lucas MOURGUES1, Rita MORCOS1, Nathalie ROCHET2, Laurence LEGROS3, Els VERHOEYEN4, Marielle NEBOUT1, Jean-François PEYRON1, Didier MARY1

1-UCA, C3M, INSERM U1065, Equipe4 « Leukemia: Molecular addictions, Resistances & Leukemic Stem Cells», Nice; 2-UCA, iBV, CNRS 7277 UMR , INSERM U1091, Nice ; 3-CHU, Département d'Onco-Hématologie, Nice ; 4-UCA, C3M, INSERM U1065, Equipe 3 «Metabolic control of cell deaths», Nice

Chronic Myelogenous Leukemia (CML) has been the first cancer to benefit from targeted therapies with Gleevec (Imatinib Mesylate). The success of this Tyrosine Kinase Inhibitor (TKI) is near 90 % but discontinuation of treatment leads to relapse in 50 % of patients. This failure is explained by the persistance of Leukemic Stem Cells (LSCs) in the medullary microenvironment : targeting those LSCs is a therapeutic challenge to succefully cure the disease.
We have previously described molecular mechanisms leading to eradication of those LSCs (Mourgues, Leukemia 2015) and by a bioinformatic approach identified a molecule, Compound AL, able to stimulate those mechanisms. Our in vitro (CML cell lines) & ex vivo (primary cells from CML patients) experiments showed strong evidences of AL in inducing cell death. Indeed, AL triggers both autophagic and apoptotic events in CML cells lines, strong decrease in terms of colony-forming units (CFU) and long-term culture initiating cell (LTC-IC), confirming it's impact on primitive stem/progenitor cells (CD34+38-) from sensible and TKI resistant patients. Moreover, we showed AL is able to affect the respiratory chain. The next goal of our project is to confirm our results in a more physiopathological model: we are developing with our collaborators a human hematopoietic niche model in immunodeficient NSG (NOD/Lt-SCID/γc-/-) mice to test the capacity of AL to reach this reconstituted human microenvironment and clean the disease. We have at our disposal a tumour bank of primary cells from LMC patients and healthy donors which are essential to have a personified microenvironment including both leukemic and stroma cells from the same patient. Thus, we want to improve the therapeutic outcome of resistant TKIs LMC patients by proposing AL in combination with TKIs.

MOURGUES L et al, Leukemia 2015

OC-3

Targeting metabolism through covalent inhibition of PKM2 in cancer treatment

Marwa Zerhouni1, Anthony Martin2, Rachid Benhida2, Stephane Rocchi*1, Guillaume Robert*1, Patrick Auberger*1

1. *C3M - Centre Méditerranéen de Médecine Moléculaire INSERM U1065, 2. Institut de chimie de Nice*

Metabolism has been recently added as a cancer hallmarks, indeed it is of great importance in the development of a tumor to sustain energy and building blocks production. Upon resistance to targeted therapy, resistant cells adapt their metabolism and more particularly glucose metabolism. To restore sensitivity to treatment, we have developed a medicinal chemistry program for metabolism targeting molecules. The compounds were screened for solubility, stability and efficiency, and lead us to the identification of HA 344 as the lead compound. Chemical biology and proteomic studies identified PKM2 as one of HA 344 covalent target. We have demonstrated that the molecule inhibited PKM2 activity and exhibited strong cytotoxicity on a wide range of cancer cell lines, including their resistant counterparts. As proof of concept, we studied HA 344 deleterious effects in vitro and in vivo on Cutaneous Metastatic Melanoma sensitive and resistant cells to BRAF inhibitors as well as on patient samples. We demonstrated that HA 344 induced cell death was p-53 mediated, caspase independent cell death. These results suggest the use of PKM2 inhibitors and more widely of metabolism modulators as potential treatment in second line for sensitive patients but more interestingly for resistant ones. Indeed, it has been demonstrated that resistance to treatment is mainly due to genetic alterations and adaptation for targeted therapy resistances, but these mechanisms of resistance are accompanied by a switch of metabolism that is not still widely explored as a treatment strategy.

Cesi et al. 2017 Henkenius et al. 2017 Maiso et al. 2015, Marrow et al. 2014, Pavlova et al, 2016, Soriano et al. 2016

OC-4

The FGF/FGFR signaling pathway: a novel therapeutic target in liposarcomas

Bérengère Dadone-Montaudié 1, Audrey Laroche-Clary 2, Aline Mongis 1, Vanessa Chaire 2, Ilaria Di Mauro 3, Renaud Schiappa 4, Emmanuel Chamorey 4, Jean-François Michiels 5, Florence Pedeutour 1,3, Antoine Italiano 6, Laurence Bianchini 1

1 Laboratoy of Solid Tumor Genetics, Université Côte d'Azur CNRS IRCAN, Nice, France; 2 INSERM U1218 Institut Bergonié, Université de Bordeaux, Bordeaux, France; 3 Laboratoy of Solid Tumor Genetics, Nice University Hospital IRCAN, Nice, France; 4 Biostatistics Unit, Centre Antoine Lacassagne, Nice, France; 5 Pathology Department, Nice University Hospital, Nice, France; 6 Department of Medical Oncology INSERM U1218, Institut Bergonié, Bordeaux, France

Liposarcomas (LPS) are aggressive adipose tissue tumors mainly represented by well-differentiated (WDLPS) and dedifferentiated (DDLPS) subtypes. Standard chemotherapy and recent targeted therapy are poorly efficient against metastatic DDLPS. Identification of new therapeutic targets is therefore mandatory. Our goal is to demonstrate that the Fibroblast Growth Factor (FGF)/FGF Receptor (FGFR) signaling pathway is involved in the tumorigenesis of WD-/DDLPS, which could be targeted by pan-FGFR inhibitors such as erdafitinib (JNJ-4275693). For this project, our research team collaborates with 3 other teams (from Nice and Bordeaux).

We performed an analysis of FGFR1, FGFR2, FGFR3 and FGFR4 expression in a collection of 400 primary tumors and in our panel of in-house LPS cell lines. Using immunohistochemistry, we observed an overexpression of FGFR1 or/and FGFR4, both in a subset of primary tumors and in all our cell lines. In univariate and multivariate analyses, we demonstrated that overexpression of FGFR1 and FGFR4 was correlated with poor prognosis.

We showed that our cell lines are sensitive to the panFGFR inhibitor erdafitinib. Exposure of the cells to erdafitinib induced a decrease in cell viability, cell cycle arrest and apoptosis. Erdafitinib treatment had a strong inhibitory effect on the ERK1/2 pathway whereas the effect on the PI3K/AKT pathway was not constant. This led us to investigate the potential synergy of erdafitinib with the PI3K/mTOR antagonist BEZ235. Synergy was observed for the WDLPS cell lines but not for the DDLPS cell lines. WDLPS and DDLPS are characterized by the systematic amplification and overexpression of MDM2. We therefore investigated the effects of the MDM2 antagonist idasanutlin (RG7388) in combination with erdafitinib. Interestingly, we found that this combination exerted a highly synergistic effect on both viability and apoptosis in all our cell lines. We are currently investigating the mechanistics of this synergy.

Conclusions

The availability of cell line- and tumor patient- derived xenograft models will allow us to validate our in vitro findings in the in vivo setting. We have shown that FGFR expression might constitute a powerful biomarker to select patients for clinical trials testing FGFR inhibitors. The availability of an early clinical trial unit in Institut Bergonié, managed by our collaborator Prof. A. Italiano, will give the immediate opportunity to transfer our data to the management of metastatic DDLPS patients.

OC-5

Studying the single-cell response heterogeneity of tumor cells to death ligand drugs

Mickael Meyer, Jeremie ROUX

IRCAN

My thesis work has been focused on understanding the molecular origins of the tumor cell response heterogeneity observed after treatment with anticancer drugs.

Indeed, intratumor heterogeneity limits the efficacy of anticancer therapies by allowing the emergence of drug resistance in tumor cells (apoptosis inhibition, autophagy, senescence) (Holohan C 2013). This cellular heterogeneity can be due to genetic diversity or a non-genetic cell to cell variability such as epigenetic changes (Almendro V 2013) and signaling dynamics (Roux J 2015). So far, the heterogeneity of isogenic cancer cells has been overlooked for technical limitations, because it required single-cell methods.

To tackle this issue, I have been using single-cell methods applied to two main objectives: (1) to determine the molecular origin of response heterogeneity from clonal cancer cells (HeLa) treated with Tumor Related TNF-related apoptosis-inducing ligand (TRAIL/Apo2L), an anticancer therapy which failed at the clinical level due to a strong therapeutic resistance; and (2) to determine the regulation of various cell death modalities commitment after treatment with combination of cancer drugs.

Firstly, by using Live-Cell Microscopy coupled with the stable expression of a probe for caspase-8 activity within our HeLa clone, we observed this activity allowed us to predict the cell fates, survival versus apoptosis, early after treatment. This predictive system gave us the opportunity to analyze the gene expression at the single-cell level of our cells after a short period of treatment decreasing the likelihood of visualizing the cellular response to focus on the differences in gene expression that cause resistance to TRAIL within HeLa cells. The Single-cell RNA-seq gave us a non-exhaustive list of genes differentially expressed between sensitive and resistant predicted cells. We are currently finishing the validation of our target genes by engineering HeLa cells lines in order to validate this new method and discover potential new biomarkers.

Secondly, we have been studying the role of p62 and RIP3K in the balance of cell death modalities after pro-apoptotic stimuli using a 4-color live cell microscopy. Here, we have been focusing on apoptosis, a non-immunogenic type of cell death induced by TRAIL treatment, and Necroptosis, a more recently described, highly immunogenic type of cell death.

OC-6

CLEC a new actor in melanoma

Henri Montaudié1,2, Laura sormani1, Bérengère Dadone-Montaudié3, Guillaume Beranger1, Caroline Pons1, Meri Tulic1, Claire Regazzetti1, Yann Cheli4, Valérie Petit5, Jean-Philippe Lacour1,2, Stéphane Rocchi1,Franck Gesbert5, Lionel Larue5*, Thierry Passeron1,2*

1 INSERM, U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), team 12, Université Nice Côte d'Azur, Nice, France 2Department of Dermatology, Centre Hospitalier Universitaire de Nice, France 3Laboratory of Solid Tumors Genetics, Institute for Research on Cancer and Aging of Nice (IRCAN) CNRS UMR 7284/INSERM U1081, Université Côte d'Azur, Centre Hospitalier Universitaire de Nice, France 4Biology and pathologies of melanocytes, Team 1, Inserm U1065, Equipe labellisée ARC 2015, C3M, Université Nice Côte d'Azur, Nice, France 5Institut Curie, PSL Research University, INSERM U1021, Normal and Pathological Development of Melanocytes, Orsay, France. University Paris-Sud, University Paris-Saclay, CNRS, UMR 3347, Orsay, France. Equipe Labellisée Ligue Contre le Cancer, Orsay, France.

Melanoma is still ranks among the most aggressive human cancers. A transcriptomic analysis from vitiligo patients has allowed to discover a new gene, CLEC, selectively and strongly expressed by melanocytes. None data on CLEC concerns melanoma. The analysis from the TCGA database reveals that patients with high CLEC expression have a significantly higher median survival than those with low expression (p=0,0125).

The first axis of my PhD project was to study the role of CLEC in vitro. Firstly, we confirmed, by western blot, a variable expression of CLEC in several melanoma cell lines and in cells extracted from patient metastases. Then, we infected the cells with a lentivirus to overexpress CLEC (OverCLEC compare to a control/OverCT) and to turn off it (ShCLEC compare to a control/ShCT). We observed that OverCLEC decreases the cell proliferation and colony formation in A375 and MeWo cells (opposite effect after knockout). We demonstrated that CLEC can recruit directly the tyrosine phosphatase SHP2 with co-immunoprecipitation assay and after generating a mutant of CLEB on its ITIM domain. Its function is mediated by this interaction. Indeed overCLEC induces a dephosphorylation of pSHP2Y542 (opposite effect with shCLEC). The mutant blocks the modulation of pSHP2Y542 and the effects observed downstream. Downstream we observed a downregulation of STAT pathway (decrease of pSTAT1,3 and 5) in overCLEC condition (opposite effect with shCLEC). The mutant of CLEC blocks these effects. Moreover, the effect observed on the proliferation is link to a slow-down in G0/G1 with an increase of p53/p21 and p27 after overCLEC. Silencing of CLEC accelerates cell cycle entry into S phase and decreases p53/p21 and p27.

The second step was to study the role of CLEC in vivo. Tumorigenic properties of CLEC were analyzed in swiss nude mice (xenograft model). In accordance with in vitro results, the tumor growth and tumor volume in the CELC group were significantly smaller than those in vehicle group and opposite observation was done with ShCLEC. Consistent with in vitro observations, overCLEC leads to decreased pSTAT3 and increased p53 level in xenograft tumor samples (opposite effect with shCLEC). Moreover, the co-immunofluorescence analysis of tumor samples revealed that CLEC represses the level of pSTAT3.

We are describing a new gene which seems to be implicated in the melanomagenesis process in acting as a tumor suppressor gene. In vitro and in vivo studies are still ongoing.

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OC-7

GAPDH overexpression in the T cell lineage in vivo promotes a T cell lymphoma

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Fundamental metabolic changes are implemented by cancer cells to meet their high energy requirements, for which they show increased dependence on glycolysis (confirmed by Warburg effect). One central enzyme of glycolytic pathway is glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that is shown to be overexpressed in many cancers and emerged, few years ago, as a key player in T cell development, function and malignancies. Here we investigated the role of GAPDH in T cell development by generating a mouse model overexpressing GAPDH in the T cell lineage. From 18 months on, clear phenotype changes were observed in these mice including: 1) splenomegaly, 2) enlarged lymph nodes, 3) lymphocytes-infiltration in the liver and bone marrow. A detailed immuno-phenotype showed an increase of strongly proliferating and clonal follicular helper Bcl6+ CXCR5+ PD1high CD4+ T cells associated with increased levels of CD95+ GL7+ germinal center B cells. Additionally, T cells from plck-GAPDH mice secreted high levels of chemokines and inflammatory cytokines compared to normal WT mice. All these features were confirmed by gene-set-expression analysis. In search for a human equivalent, we discovered that the pathological and immunephenotypic characteristics and gene expression profiling of the plck-GAPDH mice matched in great details human angioimmunoblastic T-cell lymphoma (AITL). Even the specific mutations in epigenetic modifiers typical for AITL were confirmed. Further analysis demonstrated that GAPDH-induced AITL disease depends mechanistically on activation of the non-canonical NF-kB pathway, which we also confirmed to be activated in human AITL. Targeting of this pathway with novel NF-kB inhibiting drugs combined with anti-PD1 immunotherapy increased mice survival to 70%. This combined treatment let to efficient induction of the anti-cancer immune response, proving it's efficacy. In conclusion, this new AITL mouse model, closely mimicking the human lymphoma, will help to unravel the possible origin of the disease and novel pathways implicated and will permit us to test novel therapeutic options such as immunotherapies (anti-PD1, anti-PDL1, anti-ICOS) and small molecule inhibitors of activated pathways and combinations thereof.

OC-8

Exploring the function of Ro60 complex in lipid-laden macrophages

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Ro60 is an RNA-binding protein that associates with the noncoding YRNAs to form the Ro-ribonucleoprotein complex. Ro60 is also a clinical target of the immune response in patients with systemic lupus erythematosus and Sjogren's syndrome (1). These patients mainly die of premature inflammatory cardiovascular disorders caused by accelerated atherosclerosis comorbidity (2), leading to the innovative hypothesis that the dysfunction of Ro60 in lipid-laden macrophages may enhance atherosclerosis progression.

To better understand the role of Ro60, we reproduced the lipid-laden phenotype of macrophages by palmitic acid treatment and found that pro-inflammatory and pro-atherogenic stimuli promote Ro60 binding to chromatin in primary macrophages or in THP-1, a human monocytic cell line. Interestingly, we found that YRNAs degradation allows Ro60 binding to chromatin.

Next, we performed Ro60 ChIP-seq experiments in lipid-laden THP-1 cells and control in order to find Ro60 binding sites on the chromatin. According to our biochemical data, the binding of Ro60 to chromatin increased upon PA treatment and Ro60-binding sites were distributed in different parts of the genome, including proximal promoters of genes involved in inflammatory response.

To study the function of chromatin-associated Ro60 on its target genes, we performed an RNA-seq analysis upon Ro60 knockdown in PA treated- and untreated-THP-1 cells. We found that Ro60 knockdown overall promotes a pro-inflammatory and pro-survival phenotype. By bioinformatic analysis, we found that Ro60 statistically promotes sustained transcription or induction of direct genes, including TGFBI, RAR-alpha, BMPR2, Nr4a1, and CMIP, which regulate the anti-inflammatory and pro-apoptotic response.

Altogether, these data indicate that Ro60 binding to discrete regions of the genome, including promoters of anti-inflammatory and pro-apoptotic genes, could favor the transcription of a subset of target genes that promote the anti-inflammatory and pro-apoptotic response in lipid-laden macrophages.

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OC-9

Revisiting the regulation of T cell activation by Fas receptor: critical involvement of the SHP-1 phosphatase

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Different cancer immunotherapy strategies rely on activation and expansion of cancer-specific T cells. The dissection of the molecular events that allow an optimal T-cell activation is therefore at the heart of the treatment success. Fas (CD95/TNFSFR6), a TNFR superfamily member, is a dual function signaling receptor giving rise either to pro-apoptotic or to survival functions. In T cells, Fas was described to modulate the TCR-mediated activation but the underlying molecular mechanisms have never been dissected so far. The main aim of my PhD project is to decipher these molecular cascades.

Using both a physiological coculture-based TCR dependent context and human primary T cells, I definitively pointed out a costimulatory role of Fas in TCR activation. At the molecular level, I describe (i) a constitutive association of Fas with the tyrosine phosphatase Shp-1, an interaction that could participate in maintaining the surrounding proteins such as Fas and lck in a non-phosphorylated status; (ii) an increase of the SHP-1 release to the cytoplasmic compartment upon TCR and Fas co-activation. In this context, I am currently studying the importance of (i) the Fas death domain phosphorylation, a post translational modification that we described as a switch to prosurvival signals (ii) the lck activation which initiates the TCR signaling pathway. Different axes still need to be investigated: (i) the description of Fas/SHP-1/Lck dynamics upon co-stimulation, (ii) the SHP-1 activity regulation during coactivation, (iii) the role of Fas signaling and DISC (Death-Inducing-Signaling-Complex) complex.

We believe that these results will extend our understanding of the activation-promoting T molecular signals which can significantly help to improve cancer immunotherapies.

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OC-10

Senescent cells bypass NK cell mediated immunosurveillance through a GD3 ganglioside dependent pathway

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Cellular senescence is a permanent state of cell cycle arrest that promotes tissue remodeling during development and after injury but can also contribute to (i) the decline of the regenerative potential and tissue function, (ii) inflammation, and (iii) tumorigenesis in elderly organisms (1-3).

The occurrence of age-related diseases, that evolve concomitantly with the continuous increase of human lifespan, is closely related to the accumulation of senescent cells in aging tissues without knowing precisely how and why senescent cells accumulate and how they can favor the onset of age-associated diseases. Interestingly, the immune system, through its plasticity and his complexity, is suspected to play a key role in the elimination of senescent cells in organisms through Natural Killer cells, Macrophages and CD4+ T cells (4, 5). This senescent cells immunosurveillance is expected to maintain tissue integrity and homeostasis. However, almost all experiments are performed in mouse model of oncogene induced senescence and very few data are available on the immunosurveillance of human senescent cells. The aim of my thesis is to understand how replicative senescent cells are surveilled by the immune system. My preliminary work using mass spectrometry analysis has identified a ganglioside called GD3 expressed exclusively at the senescence. The expression analysis of the ganglioside synthesis pathway by qPCR revealed that the enzyme responsible for the synthesis of GD3 (ST8SIA1) is strongly induced exactly when cells enter in senescence. Interestingly, senescent cells accumulation within tissues appears as an etiologic factor for some pathologies linked to aging as pulmonary fibrosis. In a mouse model of lung fibrosis, we revealed that senescent accumulate within the lung parenchyma which strongly express GD3.

Consistently with the strong immunoregulatory capacities of gangliosides, I observed in vivo a strong immunosuppressive function of senescent cells through the ability of senescent cells to recruit NK cells and to drive their inactivation via the triggering of the Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) Siglec7. We proposed that GD3 expression by senescent cells bypass NK cell surveillance to favor senescent accumulation and enhance lung fibrosis. Thus, the role of gangliosides in immune surveillance of senescent cells is a new avenue of research that remains to be explored in depth, especially for their implications in age-associated diseases.

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Session 2

OC-11

Heterogeneity in inflammatory osteoclasts provokes alterations in their inflammatory phenotype and bone resorption capacity

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Osteoporosis is characterized by bone destruction but also by overactivation of inflammatory CD4+ T cells resulting in enhanced osteoclastogenesis and inflammatory bone loss. Recently, our team showed that besides bone resorption, osteoclasts (OCLs) are immunocompetent cells that, depending on their origin and inflammatory status, induce different types of T cell responses. In steady state, OCLs derive from bone marrow (BM) monocytes and initiate regulatory CD4+ T cells (tolerogenic OCLs/t-OCLs), while during inflammation OCLs originate from BM dendritic cells and induce TNFa-producing CD4+ T cells (inflammatory OCLs/i-OCLs). However, the exact role of i-OCLs and underlying molecular mechanisms leading to inflammatory bone loss remain unknown.

Transcriptomic profiling of the two OCL subsets and our previous findings identified the fractalkine receptor CX3CR1 to be upregulated in ~25% of i-OCLs (CX3CR1+) while ~75% are CX3CR1neg ($p=0.001$; log2FC=3.2). To better characterize this i-OCL heterogeneity and to determine the exact function of CX3CR1+ and CX3CR1neg i-OCLs, we sorted in vitro generated i-OCLs based on their CX3CR1 expression and analysed the CX3CR1+ and CX3CR1neg i-OCLs in a comparative RNA-sequencing approach. Transcriptomic data revealed two distinct populations of i-OCLs that differ in various pathways including resorption capacity, antigen presentation and T cell stimulation. According to these findings, functional in vitro assays confirmed that CX3CR1neg i-OCLs had a higher bone resorption capacity ($p<0.0001$) and were less efficient in antigen uptake ($p=0.0026$) while expressing higher levels of molecules involved in antigen-presentation (e.g. MHC-II, $p=0.024$). Interestingly, immune suppressive pathways and genes such as PD-L1 were highly upregulated in CX3CR1+ i-OCLs ($p=0.0023$). In vitro experiments confirmed that besides reduced T cell activation ($p=0.0007$), presence of CX3CR1+ i-OCLs had a strong immunosuppressive effect on CX3CR1neg i-OCLs thereby reducing their inflammatory capacity.

These results emphasize the heterogeneity of i-OCLs and enable new insights in their inflammatory function. While CX3CR1neg i-OCLs play a major inflammatory role, CX3CR1+ i-OCLs act as immune suppressive cells controlling inflammation. A profound understanding of CX3CR1 in i-OCLs and underlying molecular pathways involved in immunomodulation will help to elucidate key mechanisms leading to inflammatory bone destruction.

Session 2

OC-12

Human Endogenous RetroVirus and Immune evasion in lung cancer

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In France, the prevalence lung cancers has reached epidemic proportions. Although critical, there are little, if any, therapeutic options to alter cancer progression. Moreover, for this cancer, metastatic diseases remain the foremost cause of cancer-related death, so it's a medical and scientific challenge, and a public health problem. Due to genetic instability, it's commonly accepted that a subpopulation of tumor acquires new aggressive properties for its dissemination, its proliferation and greater resistance to chemotherapy and immunotherapy. A better understanding of these tumor properties is a major issue to prevent this malignant progression. Our current work focus on non-small cell lung cancers, because of the lack of predictive biomarkers and therapeutic targets. We are interested on systemic inflammation in cancer development and progression, (Mantovani et al., 2008) and a scalford protein, SQSTM1 (Sequestosome or p62).

OC-13

Identification of a new pro-death axis in ccRCC patients driven by ciliogenesis and absence of VDAC1-ΔC

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Rationale: Renal cell carcinoma (RCC) accounts for about 2% of all cancers in adults with clear cell RCC (ccRCC) being the most common histologic subtype. A hallmark of ccRCC is loss of the primary cilium, a cellular antennae that sense a wide variety of signals. Loss of this key organelle in ccRCC has been characterized by loss of VHL. However, all mechanisms of ciliopathy have not been clearly elucidated

Methods: By using RCC4 renal cancer cells and patients samples, we examined the regulation of ciliogenesis via the presence or absence of VDAC1-ΔC and its impact on tumor aggressiveness. Three independent cohorts were analyzed. A Cohort A from PREDIR with 12 patients with hereditary pVHL mutations and 22 sporadic patients presenting with tumors with wild-type pVHL and mutated pVHL, a tissue samples from 43 patients with non-metastatic ccRCC who had undergone surgery (Cohort B) and 375 non-metatstatic ccRCC tumor samples produced by The Cancer Genome Atlas (TCGA) (Cohort C) were used to validation.

Results: Our study defines for the first time one group of ccRCC patients in which the hypoxia-cleaved form of VDAC1 (VDAC1-ΔC) regulates resorption of the primary cilium in a Hypoxia-Inducible Factor-1 (HIF-1) dependent manner, and one novel group in which primary cilium is reexpressed or maintained. This second group was correlated with the absence of VDAC1-ΔC in combination with maintenance of glycolysis, an EMT signature and more aggressive tumor progression but , independently of pVHL. Moreover, these patients were less sensitive to sunitinib, the first-line treatment for ccRCC, but they may be more suitable for antiglycolytic treatments and immunotherapy.

Conclusion: This study provides a new way to classify ccRCC patients and proposes potential therapeutic targets linked to metabolism and immunotherapy.

OC-14

Identification and characterization of intratumoral heterogeneity in uveal melanoma

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Uveal melanoma (UM) is the most common intraocular malignancy in adult population. More than 50% of patients develop metastases mainly in the liver. At this stage, 80% of patients die within 1 year.

Currently, histological, cytogenetic and molecular data predict the development of metastases but these studies have failed to identify therapeutic targets that could eliminate invasive populations and improve patient survival. This could be explained by the existence of rare cell subpopulations that are not detected by bulk tumor analysis. My project aims to study UMs at the single cell level to identify different cell subpopulations and make a molecular characterization of them. This approach allowed me to show the presence of a sub-population of aggressive cells, more or less represented in each UM analyzed. I also identified new genes associated with a poor prognosis, not yet described in the UM and which could constitute new prognostic markers, and/or new therapeutic targets.

Using classical biological approaches (proliferation, migration) and loss (shRNA, inhibitors) and gain (virus) of function experiments, I studied the role of the most relevant genes in the biology of UM. The mechanism of action of the most relevant candidate gene will be investigated by a transcriptomic study. Its molecular signature will be validated by genetic and/or pharmacological approaches in vitro and in vivo. The expression and role of this candidate gene and its targets will be assessed in metastatic samples of UM (staining of cells and human samples).

This project will identify new molecular markers involved in the development of UM metastases. They will represent potential prognostic markers and potential anti-metastatic UM targets.

OC-15

Factors secreted by invasive melanoma cells inhibit the contraction of lymph node fibroblasts

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Melanoma is the most aggressive skin cancer because of its high capacity to resist to treatments and its high heterogeneity. In fact, melanoma is able to switch from a proliferative signature (MITFhigh AXLlow) to an invasive signature (MITFlow AXLhigh). First metastases develop in the lymph node, a key crossroad for metastasis spreading to distant organs. The primary tumor is able to modify the microenvironment of the distant targeted tissue through the secretion of soluble factors and extracellular vesicles, leading to the pre-metastatic niche. This education of the pre-metastatic niche is necessary for tumoral invasion, proliferation and survival [1].

The lymph node is an immune tissue divided in two zones, the T-lymphocyte zone and the B-lymphocyte zone. Fibroblastic Reticular Cells (FRC) are the main population of fibroblasts in the T-lymphocyte zone, they are spontaneously contractile, produce the lymph node extracellular matrix and regulate immune cells activation and survival [2]. FRC contraction is finely regulated. During infection and lymph node swelling, dendritic cells inhibit the contraction of FRC to preserve the elasticity and architecture of the lymph node [3]. In a tumoral context, the relation between FRC contraction ability and pre-metastatic education is not known.

Using a syngeneic mice model of pre-metastatic education, we observed that the draining lymph node educated with melanoma secreted factors is bigger, more elastic, and has disorganized architecture.

My aim is to understand the effects of melanoma secreted factors on FRC contraction. Using an in vitro approach, I showed that (1) secreted factors from melanoma cell lines inhibit the FRC contraction; (2) only factors secreted by invasive melanoma cell lines, and not proliferative cell lines, inhibit FRC contraction; (3) that this inhibition is associated to modifications in cell morphology and actin cytoskeleton; and (4) inhibition of YAP (transcription factor involved in mechanotransduction and contractility of fibroblasts) and STAT3 pathway. From all melanoma secreted factors, I identify that soluble proteins with 30 to 100 kDa size are sufficient to inhibit FRC contraction, independently of extracellular vesicles.

Following this work, our goal is to formally identify the melanoma secreted factors inhibiting FRC contraction to select new predictive markers and therapeutic targets.

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OC-16

Btk toxins influence progenitor cell fate of intestinal stem cells

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Bacillus thuringiensis var kurstaki (Btk) is the most used biopesticide around the world. Btk is a Gram-positive soil bacterium. When resources are limited, vegetative Bt cells undergo sporulation, synthesizing a protein crystal during spore formation. Proteins contained in this crystal are called Cry endotoxins. Those toxins were described to be entomopathogenic to lepidopteran pests.

Our project aims to study the impacts of Btk on the gut homeostasis of the non- target organism: Drosophila melanogaster.

In the Drosophila midgut, Intestinal Stem Cells (ISCs) are required for maintenance of the proper cell composition in the adult intestine. ISC undergo asymmetric cell division that generates an ISC itself and a progenitor cell. Then, the level of Notch pathway activation in progenitor cells will commit them toward enterocytes (at high Notch activation) or enteroendocrine cells (at low Notch activation) differentiation.

Our work revealed that the number of enteroendocrine cells (EEC) increases after an intoxication by the commercialized form of Btk. We have shown that this EEC increase is dependent on the Cry toxins. We are currently deciphering the cellular mechanisms underlying this phenotype.

OC-17

The role of EFA6B in epithelial morphogenesis and its implication in breast cancer progression

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Breast cancer is the most common cancer in women around the world and the leading cause of mortality for this disease is metastasis. This process comprises of a series of complex steps in which tumor cells require the acquisition of malignant features in order to spread and invade adjacent tissues. It is of primordial importance to elucidate the factors that regulate the malignant and invasive activity of these cells. It's been reported that EFA6B a guanine nucleotide exchange factor for the Ras superfamily protein Arf6 contributes to epithelial morphogenesis by regulating the homeostasis of the tight junction and its associated apical acto-myosin cytoskeleton. Different studies have raised the possibility that EFA6B could act as an antagonist of carcinogenesis and conversely the loss of function of EFA6B might facilitate carcinogenesis. PSD4 is the gene that encodes for the expression of EFA6B in human cells. Generation of PSD4 knockout was performed in the non-tumorigenic epithelial breast cell line MCF-10A. When plated in a 3D environment composed of collagen, these cells changed their morphology developing membrane protrusions and adopting an invasive behavior. The aim of this project is to characterize the invasive properties of MCF-10A KO EFA6B cells and decipher the molecular pathways that transduce these invasive properties.

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OC-18

Functional characterization of hypoxia-regulated lncRNAs in Non-Small Cell Lung Carcinomas

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Hypoxia triggers the activation of several signaling pathways inducing a complex transcriptomic response with multiple consequences on cell survival, migration, invasion and metabolic reprogramming. This regulatory network integrates specific feedbacks and checkpoint signaling loops including epigenetic factors and non-coding RNAs. However, while a large set of miRNAs have been reported as crucial molecular regulators of the hypoxic response, the precise functional characterization of hypoxia-regulated lncRNAs is still in its infancy. Our study aimed at comprehensively characterize the whole set of hypoxia-regulated lncRNAs in lung adenocarcinoma. Using a combination of experimental profiling approaches in both tumor cell lines and patient samples as well as exploring in silico TCGA datasets, we identified a validated signature of lncRNAs correlated to i) the hypoxic status of tumors and ii) the overall survival of patients. Molecular characterization of a subset of these "hypoxa-lcnRNAs" using RNA-Seq, RT-PCR, northern blot and single molecule RNA FISH pointed to 2 interesting candidates with different subcellular localization pattern: i) NLUCAT1, a large nuclear transcript composed of 6 exons and ii) LINC01116, a short cytosolic transcript composed of 3 exons. We next used CRISPR/Cas9-mediated invalidation and transcriptomic analyses as well as RNA-pulldown approaches to functionally characterize their function and mode of action. While NLUCAT1 was mainly identified as a regulator of the NRF2-mediated anti-oxidant response with an impact on cisplatin resistance, LINC01116 was mostly associated to the regulation of cellular morphology, cell-to-cell contact and cell invasion through an alpha catenin gene network. Overall, our study strongly supports the central role of non-coding RNAs in the cellular response to hypoxia and provides the first molecular characterization of 2 hypoxa-lncRNAs contributing to an aggressive phenotype in hypoxic tumors.

OC-19

Deciphering the role of Z1 and Z3 in the pancreas

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The pancreas is an abdominal gland organized into two main tissue types: the exocrine compartment, consisting of acinar cells and a ductal system, and the endocrine tissue. Acinar cells produce digestive enzymes that are guided towards the duodenum by an organized network of ducts. The endocrine compartment is organized in highly vascularized cell clusters, termed islets of Langerhans, containing five different cell subtypes, α, β, δ, PP- and ε- cells, producing respectively glucagon, insulin, somatostatin, pancreatic polypeptide and ghrelin hormones.

Our laboratory focuses its research on Type 1 Diabetes (T1D), a chronic disease characterized by the autoimmune-mediated loss of β-cells and a consequent hyperglycemia. Given the complexity of the molecular mechanisms underlying T1D and the shortcomings of current therapies, numerous studies are undergoing with the common purpose of finding alternative approaches and therapies to this disease.

In the last decades, a great attention has been dedicated to the importance of the canonical WNT (cWNT) molecular pathway in the pancreas. Previous studies showed this pathway to be directly involved in the proper development and function of this gland throughout adulthood. Having identified members of cWNT in a screen seeking for inducers of pancreatic β-cell neogenesis, we focused on Z1 and Z3, two secreted proteins and main players of the cWNT molecular network. We thus analyzed Z1 full knock-out (Z1-/-) and Z3 conditional knock-out (Pdx1Cre::Z3cKO) mice. Combining immunohistochemistry, RNA scope, RT-qPCR and ELISA approaches with functional studies, we show that Z1 and Z3 are exclusively expressed in the acinar compartment and provide evidences suggesting a paracrine role of these proteins in regulating pancreatic hormones production and secretion. Importantly, we also show that the Z3 loss-of-function results in an increased expression of Z1. These mice display an improved glycemic control upon glucose challenge, despite a significant reduction in insulin secretion both at basal level and upon glucose stimulation. Together, our results suggest that Z1 and Z3 are key paracrine factors for proper endocrine cell function and that strategies aiming at controlling their expression could be beneficial for diabetes research.

OC-20

Role of the circadian transcription factor Krüppel like factor 10 in Nonalcoholic Steatohepatitis (NASH)

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Liver complications associated with obesity (Nonalcoholic Fatty Liver Diseases (NAFLDs)) are the leading causes of chronic liver disease. NAFLD is a spectrum of liver complications from nonpathogenic steatosis, to steatohepatitis (NASH), cirrhosis and then hepatocellular carcinoma. Although NASH prevalence is increasing worldwide, its pathogenesis is still unclear. The circadian clock is a molecular oscillator that coordinates liver physiology and transcriptome including Klf10 expression. The transcription factor KLF10 controls numerous processes involved in NAFLD progression including lipid metabolism, inflammation and cell death. Here, we investigate the development of the liver complications around the clock in a mouse model of steatohepatitis (Methionine and Choline Deficient Diet (MCDD)) and, on the other hand, the potential role of Klf10 in the development of these hepatic complications. We first show that, with exception of liver injury (ALT), hepatic steatosis (grading, Pnpla2), inflammation (Inflammatory foci, Tnfa) and the priming of fibrosis (Col1a1, Timp1) become rhythmic after 4 weeks of MCDD. Hepatic oscillations of the clock genes Nr1d1 and Per2 are unaffected whereas the peaks of expression of Bmal1 and Cry1 are phase-advanced (4.7h and 2.9h, respectively). In addition, Klf10 loses its hepatic circadian expression in WT mice fed a MCDD for 4 weeks. Hepatic steatosis and inflammation are not changed in Klf10 null (Klf10-/-) mice when fed a MCDD for 4 weeks. However, these mice display an increased liver injury, evaluated by the ALT activity, associated with a strong upregulation of Fsp27, known to promote hepatocyte death in chronic liver disease. In addition, the down regulation of Klf10 in WT mouse primary hepatocytes decreases the cell viability and enhances apoptosis (evaluated by caspase 3 cleavage and flow cytometry) in response to Tnfa/ActD. In line with these results, Klf10 hepatocyte-specific knockout mice display higher serum ALT activity compared to littermate mice when fed a MCDD for 4 weeks. All together, these results suggest that diet-induced steatohepatitis leads to a rhythmicity of hepatic steatosis and inflammation associated with a modification of the hepatic circadian clock. Furthermore, the loss of rhythmicity of Klf10 could enhance liver injury and NASH progression since KLF10 limits hepatocytes death.

OC-21

Lipid rich diet induce inflammation and thermal pain through ASIC3 activation.

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Obesity is a major risk factor for many serious disorders. It affects 13% of the whole adult population worldwide making it an important field for research. Obesity is characterized by an increased body mass index resulting from an energy imbalance between caloric intake and expenditure. This can be caused by an increased consumption of energy-dense foods such as food rich in fat, which corresponds to occidental diet. It is now well accepted that obesity induces chronic systemic low-grade inflammation, which is mediated by the Adipokines and cytokines released from the adipose tissue. This low-grade inflammation extends to other tissue leading to systemic metabolic dysfunctions. In addition, obesity was shown to be positively correlated to chronic pain regardless of other components of the metabolic syndrome. It is not yet clear how this chronic pain is initiated and what mechanisms are involved. Our study focuses on investigating the effect of obesity on peripheral sensory neurons and pain perception, followed by deciphering the underlining cellular and molecular mechanisms.

Methods

We are feeding mice with a high-fat diet to induce obesity. We are using pain behavioural experiments to measure the thermal, mechanical and chemical sensations in obese mice using radiant heat Hargreaves test, dynamic von Frey, and formalin tests. Electrophysiological approaches including patch-clamp techniques and skin-saphenous nerve preparation allowed us to study the effect of high-fat diet and obesity on peripheral sensory neurons, while molecular approaches in qPCR and Immunohistochemistry chemistry helped in investigating the changes in pro-inflammatory factors expression.

Results

In diet-induced obesity models, after 8 weeks of the regime, we show that obese mice developed a deregulation of glucose homeostasis compared to lean mice. In addition, obese mice showed a long-lasting thermal hypersensitivity once the obesity was well established, while other sensory modalities were not affected. We found an overexpression of the inflammatory cytokines in obese mice not only in the adipose tissue but also in other tissues involved in the pain pathway. We are now deciphering the role of inflammatory mediators in the nociceptive neuraxis in the thermal hypersensitivity associated with obesity.

OC-22

A root-knot nematode effector targets the spliceosomal plant machinery facilitating the giant cells formation.

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Root-knot nematodes are phytoparasites that trigger a long-lasting and intimate relationship within their host plant. To complete their life cycle, nematodes first invade the root system and secondly induce the reprogramming of 5 to 7 vascular root cells into a feeding site built-up of giant cells. Via their stylet, Meloidogyne species secrete in the host cells effectors protein synthesized in esophageal glands. In planta, these effectors manipulate some important processes such as cell cycle, cytoskeleton remodeling, plant defenses, transcriptional regulations or phytohormones signaling, leading to the formation of the giant cells. MiEFF18 (Minc18636) was identified as such a putative effector secreted to favor parasitism (Rutter et al, 2014; Nguyen et al, 2017).

The MiEFF18 effector carries a signal peptide for secretion and nuclear and nucleolar localisation signals. MiEFF18 localised into the nucleus, and particularly within the nucleolus, when transiently expressed in planta. Because MiEFF18 does not have any known function, a yeast two hybrid approach was used to search for plant partners of this effector using a tomato root cDNA library. We found the spliceosomal protein SmD1 as a high scored target of MiEFF18. We validated this interaction in planta using Bimolecular Fluorescent Complementation (BiFC). Pathogenicity tests using VIGS-silenced N. benthamiana plants and A. thaliana KO mutant lines showed that Sm protein is an essential protein involved in the nematode parasitic success. Staining of galls showed that M. incognita is not able to develop correctly in plants missing Sm. We are investigating the outcomes of MiEFF18 interaction with its target Sm, and the cellular functions, including alternative splicing, RNA quality control or PTGS modulation that may be hijacked by this effector.

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OC-23

Single-cell RNA sequencing reveals novel cell differentiation dynamics during human airway epithelium regeneration

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Background:

It is usually considered that the upper airway epithelium is composed of multiciliated, goblet, secretory and basal cells, which collectively constitute an efficient first line of defense against inhalation of noxious substances. Upon injury, regeneration of this epithelium through proliferation and differentiation can restore a proper mucociliary function. However, in chronic airway diseases, the injured epithelium frequently displays defective repair leading to tissue remodeling, characterized by a loss of multiciliated cells and mucus hyper-secretion. Delineating drivers of differentiation dynamics and cell fate in the human airway epithelium is important to preserve homeostasis.

Results:

We have used single-cell transcriptomics to characterize the sequence of cellular and molecular processes taking place during human airway epithelium regeneration. We have characterized airway cell subpopulations with high resolution and lineage inference algorithms have unraveled cell trajectories from basal to luminal cells, providing markers for specific cell populations, such as deuterosomal cells, i.e. precursors of multiciliated cells. We report that goblet cells, like secretory cells, can act as precursors of multiciliated cells. Our study provides a repertoire of molecules involved in key steps of the regeneration process, either keratins or components of the Notch, Wnt or BMP/TGFβ signaling pathways. Our findings were confirmed in independent experiments performed on fresh human and pig airway samples, and on mouse tracheal epithelial cells.

Conclusions:

Our single-cell RNA-seq study provides novel insights about airway epithelium differentiation dynamics, clarifies cell trajectories between secretory, goblet and multiciliated cells, identifies novel cell subpopulations, and maps the activation and repression of key signaling pathways.

OC-24

Comparison of unsupervised machine learning methods in localised breast cancer patients based on metabolomics signatures.

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Transcriptomic have led to the now widely used sub-type based classification of breast cancer (BC) first described by Perou in 2000. Yet there persists heterogeneity in biological behaviors within breast cancer subtypes, underlining the need to refine the taxonomy of BC. Metabolomics is a rapidly expanding field dedicated to the study of metabolism which integrates the impact of the environment on cell biology. The aim of this study was to highlight metabolomic signatures of BC obtained by 5 different unsupervised machine learning methods.

52 patients with BC and an indication for adjuvant chemotherapy between 2013 and 2016, were retrospectively included. Tumor resection specimens were analyzed. 1300 metabolomic were extracted by combined liquid chromatography-mass spectroscopy and processed using MZmine software and the “Human Metabolome” database. 5 unsupervised ML methods were used: PCA-Kmeans, Sparcl, SIMLR, Spectral clustering and K-sparse. Clinical differences between clusters and variations for every metabolite of interest were analyzed for each clustering method. Cluster separability and homogeneity was evaluated using the silhouettes method and t-sne visual evaluation.

Among the 5 clustering methods, with a partitioning optimum parameter k=3, only K-sparse and SIMLR methods generated 3 clusters with significant clinical differences, unmatched to traditional subtypes. These differences concerned: tumor stage, axillary lymph node invasion, histological grade, ki-67 proliferation index, and tumor phenotype. With a silhouette average of 0.85 and 0.91 for K-sparse and SIMLR methods respectively, those 2 methods gave the best score in terms of silhouette average and they showed a better gradient for tumor aggressiveness compared to the 3 other methods. Among them, top 50 metabolites were selected for the construction of tumor metabolome profiles for K-sparse and SIMLR, respectively. Among selected metabolites we found a significant increase of L-phenylalanine and L-methionine along with a significant decrease in glutathione and glutamate in the cluster associated with poorer histopronostic factors.

Unsupervised ML methods generate heterogeneous results when applied to metabolomics data extracted from BC patients. K-sparse and SIMLR were able to identify three different groups based on tumor metabolome. Tumors with the worst histopronostic factors seemed to present higher concentrations of protienogenic amino-acids

OC-25

Age-dependent remodeling of neuronal RNP granules

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Nascent mRNAs complex with RNA binding proteins (RBPs) to form highly dynamic, phase-separated organelles called ribonucleoprotein (RNP) granules. Neuronal RNP granules play critical roles in mRNA storage and processing, stress response, synaptic plasticity and neuronal function. Regulation of their dynamic properties is crucial, as progression of end-stage neurodegenerative diseases is characterized by the accumulation of toxic aggregates rich in RBPs. Such aggregates are also visible upon physiological aging, where they are linked to neurodegeneration and impaired motor abilities and cognition. Although recent in vitro work has proposed that aggregation of RNP components is linked to changes in the material properties of normally dynamic assemblies, the cellular processes regulating such changes during physiological aging are unknown.

In the laboratory, we study neuronal RNP granules that are characterized by the presence of IMP, the Drosophila ortholog of ZBP1. These granules assemble in the cell body of CNS neurons and are transported to axons in specific populations. They contain profilin mRNA, a direct target of IMP encoding a regulator of the F-actin cytoskeleton. To study how aging impacts on neuronal RNP granules, we have analysed IMP neuronal RNP granules in flies of increasing age. Strikingly, an increased clustering of IMP molecules into granules was observed upon aging. The large IMP granules observed in aged flies were dynamic, contained profilin mRNA, and did not colocalize with aggregation markers, suggesting that they do not correspond to protein aggregates. Interestingly, increased clustering of IMP was associated with an increased clustering of profilin mRNA, recruitment of translational repressors such as Me31B and Dcp1 and the specific inhibition of profilin reporter translation. Through a candidate-based RNAi screen carried, we showed that downregulation of PKA activity reversed the clustering of IMP granule components observed upon aging. Together, these results for the first time demonstrate that the activity of a specific signalling pathway modifies the properties of neuronal RNP granules during aging, increasing the translational repression of associated mRNAs. Given that downregulating the PKA pathway was previously shown to rescue age-related memory impairment in Drosophila, our work thus establishes an interesting link between changes in neuronal RNP granule properties and memory retention.

1) Patel et.al., 2015, 2)Yamazaki et.al, 2007

OC-26

Impact of a Fragile X Syndrome missense mutation on the neuronal function

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Fragile X syndrome (FXS) is the most frequent form of inherited Intellectual Disability (ID) and a leading monogenic cause of autism. FXS results from mutations within the FMR1 gene causing the loss-of-function of the Fragile X Mental Retardation Protein (FMRP). FMRP is an RNA-binding protein that transports several mRNAs along dendrites to the base of active synapses for local translation in an activity-dependent manner. In FXS, the loss-of-function of FMRP leads to a hyper-abundance of immature dendritic protrusions, resulting from abnormal translation events at the synapse. Such defects lead to altered postsynaptic maturation and pruning and, consequently, synaptic communication and plasticity deficits. We recently demonstrated the activity-dependent SUMOylation of FMRP in vivo, which is critical to the neuronal function. Interestingly, several FMR1 missense mutations identified in FXS patients lead to amino-acid changes that are close to the active SUMO sites of FMRP. This raises the exciting hypothesis that these mutations directly affect the SUMOylation state of FMRP and, consequently, its function, leading to FXS. Thus, we successfully engineered a Knock-In mouse model expressing one of these FMR1 mutations. Using this unique FXS model and a combination of state-of-the-art approaches, we are currently dissecting the impact of this FXS mutation on the FMRP function with particular attention to its activity-dependent SUMOylation and synaptic regulatory role.

OC-27

Functional characterization of new genes implicated in Early Onset Schizophrenia and Autism

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Early Onset Schizophrenia (EOS) is a psychiatric disorder characterized by a wide range of symptoms such as delusions, hallucinations and abnormal social behavior [1]. This disease is frequently accompanied by Autism Spectrum Disorders (ASDs) and Intellectual Disability (ID), which are believed to share a common genetic background with EOS [2; 3]. During this study, the Whole Exome Sequencing (WES) of 9 TRIO (child and the two parents belonging to a cohort of 40 families sharing a similar phenotype) was performed in order to find novel genes that could be involved in the pathogenesis of EOS and ASD. Probands were diagnosed with EOS, which in some cases was combined with other phenotypes such as ASDs or ID. We found some very rare variants that have a putative pathological impact on the phenotype of the patients according to in silico analysis. In particular, one variant was identified in the GENE1 that is a distant member of the CAMK group of serine/threonine kinases. The members of this family participate in the regulation of calcium homeostasis that was shown to be altered in patients with schizophrenia. To further evaluate the pathological impact of the identified variants, we are using different approaches that depend on the characteristics and potential functions of the candidate gene. For GENE1 we created a cellular model in the SH-SY5Y cell line (a neuron-like line) by the CRISPR-Cas9, which is a technique that enables the introduction into the cells the deletion of 22 nt within a splicing region mimicking the potential mutation found within the patient and we obtained the mutated cell lines both in heterozygous and homozygous states. After validation of the presence of the mutation by western blot and RT-qPCR, we performed a proteomics analysis comparing WT and mutant cell lines and we identified some deregulated pathways such as calcium homeostasis and signaling, energy metabolism, maintenance of cytoskeleton, neuronal transmission and synaptic function. Interestingly most of these pathways have been previously associated with Developmental Brain Disorders [4].

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OC-28

A-eta-alpha: another app protein with impact on hippocampal synaptic function or why it is important to have an overview before we try to cure

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Alzheimer's disease (AD) has increasing prevalence in aging population with significant impact in economy. Synaptic dysfunction plays a central role in AD, since it drives cognitive decline. Genetic studies highlight the amyloid precursor protein (APP) role in AD. Thus, deciphering the role of the different APP fragments in synaptic function is crucial to fully understand AD etiology. In Willem et al. [(2015) Nature, 526: 443–447] we described a new APP processing pathway producing amyloid-η (Aη) peptides. We could demonstrate that the Aη-α peptide, the longest form of Aη produced by η-secretase and α-secretase cleavage, is detrimental for hippocampal function as it impairs biological mechanisms underlying memory formation. Going beyond these initial observations, we now performed an extensive analysis of its action on various parameters of plasticity at the CA3-CA1 synapse.

To comprehensively investigate the actions of Aη-α at the excitatory glutamatergic synapse, we performed ex-vivo field electrophysiology, together with pharmacology, on hippocampal slices of adult mice. Electrophysiology protocols were designed to discriminate Aη-α action at the pre-, post-synapse and plasticity threshold.

Our results show that both synthetic and cell-expressed recombinant Aη-α impacts synaptic plasticity at low nanomolar concentration, without affecting basal synaptic transmission. In particular, while Aη-α does not perturb pre-synaptic short-term plasticity, it significantly modifies the induction threshold of post-synaptic long-term depression and long-term potentiation.

These results show that Aη-α can acutely influence neuronal plasticity via alterations of mechanisms at the post-synapse. We are currently testing how Aη-α actions at synapses translate into changes in cognition.

Willem M, Tahirovic S, Busche MA, Ovsepian SV, Chafai M, Kootar S, Hornburg D, Evans LD, Moore S, Daria A, et al. eta-Secretase processing of APP inhibits neuronal activity in the hippocampus. Nature. 2015;526(7573):443–7. doi: 10.1038/nature14864.

OC-29

Negative dominance: a novel mechanism in SCN2A mutations causing Autism

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The SCN2A gene encodes for the α-II subunit of voltage-gated sodium channels Nav1.2. These channels are mainly expressed in excitatory neurons and play an important role in the initiation and propagation of action potentials early in the postnatal development.

Mutations on the SCN2A gene are responsible for different pathologies such as BFNIS (Benign Familial Neonatal Infantile Seizures), EE (Epileptic Encephalopathies with infantile onset), Ohtahara Syndrome (OS, an epileptic encephalopathy with neonatal onset), autism, schizophrenia and intellectual disability.

Previous studies have been performed in order to disclose phenotype/genotype relationships. They showed a loss of function of Nav1.2 channels for mutations causing autism and EE, and a gain of function for mutations causing BFNIS and OS. However, the detailed differential mechanisms giving rise to different pathologies remain unclear. To better understand these mechanisms, we studied the functional effects of 22 SCN2A mutants expressed in neocortical neurons in primary culture.

We observed that mutations causing autism or EE lead to a clear Nav1.2 loss of function, whereas most of the mutations causing OS lead to a gain of function.

To better disclose the physiopathological mechanisms of these mutations, we performed the analysis co-expressing WT and mutant channels. Our data show that the current density of WT channels is significantly reduced only in the presence of the mutations responsible for autism. These results are in accordance with a dominant negative effect of the mutants on WT channels. As it has been recently shown that the α-subunits of voltage-gated sodium channels can interact and form dimers (Clatot et al, 2018.,Nature Communications), we used different strategies to prevent this interaction. Using conditions that remove the interaction between two α subunits, the dominant negative effect of mutant channels on WT channels is abolished, indicating that the interaction between two α-subunits is essential to observe the dominant negative effect. Our data bring unique information about the physiopathological mechanism of mutations of SCN2A responsible for autism and disclose that negative dominance plays a key role in this mechanism.

Clatot et al, 2018.,Nature Communications

OC-30

Polyunsaturated phospholipid incorporation in host membranes attenuate bacterial invasion

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Polyunsaturated phospholipids (PUPLs) are important components of cellular membranes notably endothelial cells. The chemical structures and physical properties of long-chain polyunsaturated fatty acids make PUPLs more flexible than other PLs and thereby facilitate membrane deformations involved in endocytosis. Here, we have investigated the effects of PUPLs on two distinct bacterial invasion processes. One is the formation of transendothelial cell macroaperture (TEM) induced by bacterial toxins. TEMs represent a novel way for bacterial extravasation through the endothelium. We found that PUPLs restrict the widening of TEMs leading to the opening of tunnels of smaller size. Cell invasion is also studied in a model of uropathogenic Escherichia coli (UPEC) entry into host cells. UPEC can invade host cells and form infectious reservoirs thought to promote recurrent infections. We found that PUPLs inhibited the invasion of UPEC. Together, our results shed light on the importance of fatty acyl chain composition of PLs in actin-driven large-scale membrane deformations that underlie two critical invasive steps in bacterial infection.

OC-31

Contributions of aminopeptidase A and dipeptidyl peptidase IV to N-terminal truncations of Aß peptide : in vitro, ex vivo and in vivo approaches

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A defining characteristic of Alzheimer's disease pathology is the presence of extraneuronal plaques composed of aggregated beta-amyloid peptides (Aß). However, the vast majority of clinical trials centered on Aß by either blocking their production or neutralizing them, once produced, have failed. Thus, the contribution of Aß-derived fragments generated by secondary cleavages could have been underestimated. Our hypothesis is that exopeptidases responsible for secondary cleavages occurring in N-terminal region could contribute to the pathlogy. The implication of two exopeptidases, namely aminopeptidase A (APA) and dipeptidyl-peptidase IV (DPPIV) in the truncation of Aß peptide has been examined. Using mass spectrometry approaches, we show that DPPIV releases the two first residues of Aß40, thereby yielding Aß3-40. In organotypic slices, we establish that inhibition of endogenous DPPIV increases full length Aß40. Further, incubation of slices with synthetic AßpE3-42 modifies dendritic spines morphology. Finally, in vivo, oral administration of a selective APA inhibitor (RB150) reduces AP activity and decreases of pE3Aß42 in both insoluble and soluble fractions. Interestingly, pE3Aß42 species were observed in glial cells, suggesting a communication between neuronal and glial cells. Taken together, this study provides the first histological and biochemical evidence of pE3Aß42 presence in 3xTgAD mice and identifies APA and DPPIV as potential pathogenic exopeptidases. The development of shRNAs lentiviruses targeting endogenous APA and DPP4 will allow us to establish the influence of APA and DPPIV in AD onset and progression in AD mice models.

OC-32

KCNE1 activates the voltage-dependent and calcium-activated chloride channel TMEM16A

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Ancillary (?) subunits constitute one of the most important sources of diversity of electrical signals in cells. Although they cannot induce native currents per se, they associate with pore-forming (α) subunits of ion channels and modulate their pharmacological and biophysical characteristics. Their remarkable importance is reflected by the large number of diseases related with them when they are impaired, such as muscular troubles and cardiac arrhythmias. Although promiscuity between ancillary subunits and ion channels has been previously shown, no ?-subunit has been identified to regulate permeation of two different ions. The present work shows that KCNE1, typically known as an ancillary subunit of the voltage-gated K+ channel KCNQ, can also regulate TMEM16A, a voltage-dependent and Ca2+-activated Cl- channel. By combining patch-clamp and Single Molecule Pulldown assays we have observed that KCNE1 interacts physically with the channel, inducing Cl- flow in absence of Ca2+. We have also found that the extracellular domain of KCNE1 is necessary for TMEM16A activation, whereas phosphorylation by PKC of the intracellular domain greatly enhances its conductance. Such findings are in agreement with other studies showing that cRNA injection of KCNE1 or incubation with KCNE1 peptides not only activates endogenous K+ (KCNQ1) channels in Xenopus laevis oocytes, but also Cl- channels. Here, we have identified TMEM16A as the Cl- channel activated by KCNE1 and found that they constitute a complex carried endogenously by proximal tubule epithelial cells that may be critical for Cl- secretion in kidney. Our results break with dogma that one ancillary subunit can only regulate a single ionic conductance and address to them a boarder role in cell physiology by controlling the flow of more than one ion.

OC-33

Role of transcription factor Otx2 in adult retina photoreceptors

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Photoreceptors (PRs) are the cells responsible, in the retina, to convert light in electric signals, that are sent to the brain after a sophisticate elaboration. Their development and specification are under control of a complex gene network in which a key role is played by the transcription factor Otx2. Even if PRs keep Otx2 expression in adult stage, together with the neighbour Retinal Pigmented Epithelium (RPE) and bipolar cells (BPs), the role of this protein at this stage is not yet fully characterized. Recently our laboratory showed that, depleting Otx2 expression in these three retinal compartments, induces PRs specific degeneration, a phenotype that is interestingly recapitulated when the protein is abolished only from the RPE. On the other side, in the same full retina KO context, Otx2 re-expression just in the RPE, is sufficient to maintain alive Otx2-null PRs.

Those observations open two main questions, actually under investigation during this PhD fellowship:

- What is the role of Otx2 in the PRs?
- How Otx2 expression in RPE is sufficient for Otx2-depleted PRs?

To answer the first question, we induce Otx2 ablation specifically in the adult PRs, in a particular transgenic mice line, analysing later at different biological levels (RNA-seq, cell-viability and identity, PRs functionality); for this part of project, preliminary results have confirmed a marginal role of Otx2 in the maintenance of the adult PRs, even if further experiments will tell us if those PRs are functional.

For the second question we speculated, according with some recent publications, a direct transfer of Otx2 from RPE to PRs. To test this interesting hypothesis, we inject in the retina AAVs, driving tagged-Otx2 expression specifically in the RPE and then we check if the tagged protein is up-taken by PRs. Even if a direct prove is still under missing, we have preliminary data that could fit with our hypothesis.

Should we have success to show a transfer of Otx2 from the RPE to the PRs, we could also imagine this neuroprotective process as a compensatory mechanism to help Otx2-depleted PRs to be retained during life. Moreover, understanding the roles of endogenous and exogenous Otx2 in the PRs would be of great impact to set up new gene-therapy approaches for retinal diseases.

OC-34

Morphological and electrophysiological characterization of layer V subcortical projection neurons in a transgenic mouse model of fine motor skill impairments

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The mammalian neocortex, responsible for high brain functions such as voluntary movements and sensorimotor integration, is subdivided into functionally distinct areas and composed of six layers. This peculiar structure mirrors the function of each area: the primary motor cortex (M1), that controls the execution of movement, is largely populated by layer V output neurons, whereas the primary somatosensory area (S1), which processes sensory stimuli, includes a broad population of layer IV integrative neurons. Cortical neurons connect themselves up into dynamic networks that generate and maintain stable activity patterns via a fine-tuning of intrinsic excitability, synaptic strength and network excitability and inhibition.

We have previously shown that loss of function of the COUP-TFI (Nr2f1) gene in the cerebral cortex leads to altered areal organization and impaired development of layer V projection neurons (PNs). This results in altered voluntary movements (1) and reproduces some of the symptoms observed in human patients carrying mutations in the NR2F1 gene (2). However, it is still unknown whether these mis-specified PNs are able to mature correctly and to project to their proper targets. By using COUP-TFI cortical conditional mutant mice (3) crossed with the reporter Thy1-eYFP-H mouse strain (4), in which layer V PNs are strongly labeled, we were able to assess the structural and functional integrity of layer V PNs in normal and pathological conditions.

Comparing the 3D spatial distribution of YFP+ axons in the pontine nuclei, we found that COUP-TFI-deficient layer V corticopontine (CP) axons are more peripherally distributed than their control counterpart, with lower representations in centrally located regions that normally receive dense projections from S1. This suggests that COUP-TFI acts on the topographic CP projection map during development. Moreover, by analyzing the morphological and electrophysiological properties of layer V PNs, we observed impaired intrinsic excitability and basal dendritic arbor complexity at juvenile stages, as well as reduced dendritic spine density at adult stages.

Our data show that COUP-TFI plays a key role in directing structural and functional maturation of layer V PNs during development. Moreover, we show that early modifications in cortical arealization are reflected in later spatial organization of CP projections and COUP-TFI is directly involved in the establishment of topographically organized neural networks.

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OC-35

Conserved gating of vertebrate and invertebrate two-pore K+ channels

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Mutations that modulate the activity of ion channels are essential tools to understand the biophysical determinants that control their gating. Here, we reveal the conserved role played by a single amino acid position (TM2.6) located in the second transmembrane domain of two-pore domain potassium (K2P) channels. Mutations of TM2.6 to aspartate or asparagine increase channel activity for all vertebrate K2P channels. Using two-electrode voltage-clamp and single-channel recording techniques, we find that mutation of TM2.6 promotes channel gating via the selectivity filter gate and increases single channel open probability. Furthermore, channel gating can be progressively tuned by using different amino acid substitutions. Finally, we show that the role of TM2.6 was conserved during evolution by rationally designing gain-of-function mutations in four Caenorhabditis elegans K2P channels using CRISPR/Cas9 gene editing. This study thus describes a simple and powerful strategy to systematically manipulate the activity of an entire family of potassium channels

OC-36

Characterization of bone extracellular matrix produced by recql4-deficient osteoblaststle

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Bone undergoes constant remodeling where osteocytes, cells acting as mechanosensors, coordinate the function of osteoclasts, resorbing old and damaged bone, and of osteoblasts (OB), synthesizing new mineral matrix. Any disruption of the equilibrium between these processes could lead to a pathological bone condition. Our laboratory is studying the recql4-/- murine model. RECQL4 is a DNA helicase involved in genomic stability and its dysfunction has been associated with cellular senescence. Our results from micro-CT analysis of recql4-/- and recql4+/- (control) mice show that mutant mice exhibit a decrease in bone mineral density with age. This is reminiscent of the premature bone aging phenotype observed in patients with recql4 mutations. To decipher the mechanisms underlying bone loss in our model and in patients, we planned to investigate interactions between the mineralized ECM and bone cells. Primary OB were isolated from recql4-/- and recql4+/- mice and used to synthesize bone ECM in vitro. Protein and mineral composition and ultrastructure of those ECM were examined. Bone matrices produced by recql4-/- OB tend to be less mineralized than those from control OB, although organic component of recql4-/- matrices is stiffer. 204 out of 1363 proteins identified by proteomic analysis have a significantly different level of expression between control and mutant. Ingenuity Pathway Analysis on our protein set did not reveal any significantly modified pathways, but helped select 6 candidate proteins. All of them are known to be involved in osteogenesis regulation. Literature also suggests that dysfunction of 5 of them could lead to a phenotype similar to the one observed in our mouse model. Currently we are analyzing the expression level of those proteins by complementary approaches.

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OC-37

VapBC toxin-antitoxin modules of Sinorhizobium meliloti : actors of the nitrogen-fixing symbiosis

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Background: The symbiotic interaction between Sinorhizobium meliloti and the model legume Medicago truncatula leads to the formation of new root organs, the nodules, where differentiated bacteria reduce the atmospheric nitrogen into ammonium.

Objectives: To better understand the intracellular lifestyle adaptation of bacteria during symbiosis, we examine the role of S. meliloti VapBC Toxin-Antitoxin (TA) systems. These TA systems are composed of a stable toxin (VapC) and a labile antitoxin (VapB) inactivating the toxin. In response to a signal, antitoxin degradation by bacterial proteases releases the toxin, acting as a post-transcriptional regulator due to its RNase site-specific activity.

Methods: Importance of VapBC modules was studied by phenotyping the interaction between Medicago and bacterial mutants deficient in the VapC toxin on their ability to nodulate, differenciate, fix nitrogen and persist in nodules. Lastly, identification of the consensus site of cleavage of representative VapC toxins by a RNA-seq method has been undertaken.

Results: Infection of M. truncatula with vapC mutants show that 4 mutants among 11 have an altered symbiotic phenotype: defect in root infection, early or delayed nodule senescence. This study demonstrates the overall importance of TA at all steps of symbiosis, making them essential actors in the plant-microbe interaction fitness. RNA-seq approach will contribute to identify the RNA targeted by specific VapCs, and to connect a defined symbiotic function to a specific VapBC module.

OC-38

The Arabidopsis Receptor-Like Kinase IOS1 links filamentous pathogen attack to Endoplasmic Reticulum stress

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To sense the environment, plant cells possess more than 200 plasma membrane-spanning receptors, which are composed of extracellular leucine-rich repeats (LRRs) and an intracellular kinase domain. We previously identified a member of this family of receptors in Arabidopsis, which contributes to the infection success of filamentous, biotrophic pathogens such as oomycetes (Hok et al., 2011; Hok et al., 2014). The extracellular region of the receptor-like kinase "Impaired Oomycete Susceptibility 1" (IOS1), is composed of LRRs and a domain, which shares similarities with malectin from animals. Animal malectins bind carbohydrates and monitor proper folding of glycoproteins in the endoplasmic reticulum (ER), where they interact with ribophorins of the Oligosaccharyltransferase (OST) complex. We observed retention of IOS1 in the ER, which is mediated through the malectin-like (ML) domain. We show that the extracellular ML domain of IOS1 interacts in yeast with the plant ribophorin HAP6 at the ER membrane. By mutant analyses, we assessed the roles of IOS1 and HAP6 in responses to both downy mildew infection and ER stress. qRT-PCR analysis showed that the Unfolded Protein Response (UPR) signaling was affected in both IOS1 and HAP6 mutants. Our data suggest that the individual domains of IOS1 convey dual functions to the receptor in different subcellular compartments. Downy mildew infection triggers the UPR in the ER of Arabidopsis cells. A modulation of this response by the ML domain of IOS1, similar to the function of malectin in animal cells, might then promote the infection success of the pathogen.

OC-39

Cross-talk between aphid facultative symbionts and nitrogen fixation symbiosis in the aphid and legume interaction

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Plants, animals, and humans live in a microbial world and a wide range of interactions with microbes have evolved in host-specific symbioses, which are usually beneficial for both the host and the microbe(s). These symbiotic interactions include aphids that live in associations, obligatory and facultative, with certain bacteria. Aphids are major agricultural pests through their plant feeding damage and virus transmission and because of their asexual reproductive capacity. Aphid growth and impact on plant fitness are strongly influenced by the interaction with other partners such as the facultative endosymbionts (SS) they harbor. Leguminous are important agricultural plants attacked by aphids, notably pea aphid (Acyrthosiphon pisum). These plants are able to reduce atmospheric nitrogen in ammonia to satisfy the plant nitrogen need via symbiosis with the soil Rhizobium bacteria. This symbiosis takes place in a new plant organ, the root nodule. In this context, it is important to know whether and how the presence of different facultative symbionts in the pea aphid and in the host plant modulate the legume-aphid interaction and vice-versa.

In order to address this question, we have evaluated the effect of the Nitrogen-Fixing Symbiosis (NFS, inoculated plant) compared to watering with nitrates (non-inoculated plant) in Medicago truncatula, a leguminous plant model, on the development and growth of five lines of the aphid Acyrthosiphon pisum. These lines are either deprived of SS or host only one SS (Hamiltonella defensa, Regiella insecticola natural or artificial, Serratia symbiotica) in a YR2 clone background. We also tested for an effect of infestation by the aphid lines on the growth of inoculated vs non-inoculated plants, and on the symbiotic nitrogen fixation. We have also analyzed the M. truncatula defense response to the different aphid lines by expression analysis of plant defense gene markers. We showed that NSF reduces aphid fitness independently upon the aphid lines. The infection by the majority, but not all, of the aphid SS decreases significantly plant nitrogen fixation efficiency. Finally, a specific defense response was observed in the nitrogen-fixing plants compared to nitrate-fed plants independently of the aphid lines. Overall, our results demonstrate that plant-aphid interactions is influenced both by the plant and the aphid symbiotic partners.

OC-40

Regulation of senescence-specific proteases during the nitrogen-fixing symbiosis in Medicago truncatula.

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The Legume-Rhizobium symbiosis is time-limited due to the initiation of a senescence process, leading to a complete degradation of bacteroids and host plant cells. The increase in proteolytic activity is one of the key features of this process. A papain family cysteine proteinase, MtCP6, has been evidenced to be involved in the senescence process of Medicago truncatula nodules (Pierre et al., 2014). Corresponding gene induction was observed during both developmental and stress-induced nodule senescence.

In order to decipher the senescence-signalling pathway in symbiotic nodules, we focus on the analysis of the cis-regulatory elements present on the MtCP6 gene promoter (ProCP6) by constructing series of deleted promoter fragments. A search for specific transcription factors (TFs) is undergoing in order to fully understand such specific regulation of cysteine proteases genes (CPs). This work will lead to a better understanding of the regulation of cysteine proteases, and to the characterisation of transcription factors involved in the regulation of developmental-induced senescence in the nitrogen-fixing symbiosis.

Pierre, O., Hopkins, J., Combier, M., Baldacci, F., Engler, G., Brouquisse, R., . . . Boncompagni, E. (2014). Involvement of papain and legumain proteinase in the senescence process of Medicago truncatula nodules. New Phytologist, 202(3), 849-863.

OC-41

Increasing crop diversity in space and time at a filed scale to promote sustainability of agricultural systems

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The importance of plant richness on natural enemies presence, biodiversity and efficiency has largely been studied and proved these last decades. The planting and preserving non-crop plants or manipulating crops richness in field are techniques that have proven their efficiency. However, the impact of crop richness continuity in space and time on pests and natural enemies at a landscape scale remains poorly studied. In this two years study, we assessed the effect of crop richness on pests and natural enemies in a field experiment at the Langfang experimental station in China. The increase of crop species within a plot have been proven to be positive for polyphagous pests and negative for monophagous ones. The effect on natural enemies is varying regarding the crop period, the evolution of biomass and flower availability in the plots and the natural enemies guild. However, the differential succession in space and time of crops in the polycultural system was highly stimulating the spillover of the ladybeetles with no similar effect in monocultures. Significant relation between pests and natural enemies were increased in polyculture in the case of lacewings and spiders. The time spent by ladybeetles, lacewings and spiders on a specific crop in the polycultural systems was longer than in the monocultures. However, as a clear reduction of pest abundance or a clear increase in natural enemies presence could not be established, repeating this study in order to reduce the year effect might be of great interest in order to see if the tendencies seen can be confirmed or not.

OC-42

The role of CCL17 in the murine brain development and behavior

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Background.

Maternal infection during pregnancy is an important risk factor for the development of autism spectrum disorder (ASD) and requires further study with the goal to ultimately improve diagnosis and therapy outcome. Pre-clinical studies have shown that infection of pregnant mice with influenza virus (or injection of the viral mimic poly(I:C)) induces ASD-like behaviours, in association with altered profiles of immune markers such as cytokines and chemokines. Starting from recent clinical discoveries by our team, we set out to investigate the role of CCL17 in brain development and function in the murine maternal immune activation (MIA) model of ASD.

Methods.

The MIA model was obtained by injecting 2 mg/kg of poly(I:C) intraperitoneally into pregnant dams at embryonic day 10.5. To check for behavioral alterations, the resulting male pups were subjected to a panel of sensorimotor tests and ultravocalisation recordings (USVs) during the first two weeks, as well as tests specific for autism behaviours in adulthood. To confirm the phenotype with molecular cues, we analysed the level of CCL17 in serum samples.

Findings.

MIA pups exhibited an increase in the number of ultrasound vocalisations at postnatal day six, indicating impaired communication. In two-month old adult MIA mice, we found decreased prepulse inhibition of startle, a sign of reduced sensory gating. We also found increased distance travelled in the open field, both in two weeks-old and adult MIA mice, suggesting hyperactivity. We later measured the serum levels of CCL17 in pups and adult MIA mice and found altered circulating levels of CCL17 in young animals.

Interpretation.

Preliminary data suggest that increased serum levels of CCL17 are associated with abnormal behavior in mice. Due to the heterogeneity of the behavioural data, we are currently working to strengthen the model by improving its reproducibility in behavioural tests.

OC-43

Environmental and genetic impact on telomere size dynamic in 3 coral species across the Pacific Ocean

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Telomeres are composed of short repeated DNA sequences located at the end of linear eukaryotic chromosomes. Since the replication of DNA extremities is incomplete, telomeric DNA shortens at each round of the cell's division, interestingly, this shortening rate can be modulated by environmental stress. Excessive telomeres shortening will result in unprotected DNA's ends thus leading to senescence and/or apoptosis but in the meantime preventing cancer by limiting cell proliferation (1,2). Telomere homeostasis is therefore a key mechanism which leads to premature aging or cancer when disrupted (3,4). Our knowledge on the contribution of environmental changes on telomere length (TL) variability remains at its infancy, as well as the diversity of telomere maintenance mechanism existing in other organisms. Mostly in some species which exhibit extreme lifespan without increasing their sensitivity to cancer proliferation such as naked mole rats, bats, tortoises, corals (5) … In this regard reef corals are an interesting but yet not investigated model systems (6). They are long-lived fixed colonial invertebrates that display symbiosis within their gastrodermal cells hosting photosynthetic micro-algae. Furthermore, they host a still largely unknown world of associated bacteria, viruses, and other protists, forming a complex symbiocosm or 'holobiont' (7). Taking advantages of the TARA-Pacific expedition (8) sampling I am studying 3 different coral species (Porites lobota, Pocillopora meandrina, Millepora platyphylla), that were sampled over 32 island systems in the Pacific Ocean. TL is assessed using differential Southern Blot hybridization since the animal and its symbionts have different telomeric repeat sequences (TTAGGG for the animal and TTTAGGG for the algae) (9). First results show a clustering of phylogentic groups with the same average animal TL for Porites lobota and Pocillopora meandrina, while Millepora platyphylla's is significantly higher. There is no obvious correlation between the symbiont and the coral TL mean in any of the 3 species. But it appears that within one island there is an interspecific difference in TL, as well as TL variation between islands within each species that remains to be explained regarding genetic data and environment.

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OC-44

Defect of mitochondrial pseudouridylation : Identification of a novel mitochondrial disease gene ?

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Mitochondrial diseases (MD) are the most frequent metabolic disorders. Their diagnosis is very difficult because of both their clinical and genetic heterogeneity. Even though a growing list of nuclear genes are discovered as involved in MD, in one patient out of two the responsible gene is unknown. I recently showed that the use of very large panels does not improve diagnostic yield of MD (Plutino et al., 2018). Whole exome sequencing has emerged as the preferred method for identifying Mendelian disease genes and by using this method, we found a homozygous variant in the PUSL1 gene in a young boy presenting with a progressive encephalopathy and signs of mitochondrial dysfunction. PUSL1 encodes a pseudouridine synthase 1-like. This protein has not been characterized yet, but is a member of the pseudouridine synthases family corresponding to enzymes responsible for isomerization of uridine to pseudouridine in cellular RNAs. Interestingly, PUSL1 is a paralog of PUS1, encoding the pseudouridine synthase 1, which has been involved in Myopathic Lactic Acidosis with Sideroblastic Anemia (MLASA), a severe mitochondrial disease. Segregation study and in silico analysis suggest a deleterious effect of the PUSL1 variant identified in our patient. My work aims to confirm or refute the pathogenic nature of the identified variant and to better understand the role of PUSL1 in mitochondrial translation by using fibroblasts of the patient.

OC-45

Mechanical forces during filamentous growth of a human fungal pathogen

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Physical forces appear to be critical for host tissue penetration by the human fungal pathogen *Candida albicans*, as well as its escape from host immune cells (1). *C. albicans* filamentous growth and its ability to invade or colonize solid surfaces, such as medical implants and human tissues are critical for its pathogenicity. With the goal of determining the biophysical properties of *C. albicans* hyphae, we have generated PDMS microchambers (2, 3), in which *C. albicans* are entrapped and subsequently monitored filamentous growth. Furthermore, we have altered the stiffness of these microchambers to mimic that of human cells and tissues and have characterized surface and invasive growth, using time-lapse microscopy. We observed a decrease in the percentage of invasive filamentous growth with an increase in PDMS stiffness. Strikingly, there appears to be a stiffness threshold where hyphae no longer are able to penetrate. This threshold is likely to be at the growth-stalling force, a value, which is balanced by the cell turgor pressure (2). When unable to penetrate the PDMS, hyphae bend upon extension, eventually filling the microchamber. We have followed filament invasion within the substrate and show that they extend with a reduced rate compared to surface growing cells. There is a further reduction in extension rate with an increase in PDMS stiffness. Additionally, we have measured a significant increase in cell diameter concomitant with a decrease in cell compartment length during invasive growth, yet, compartment volumes are indistinguishable to that of surface growing cells. These results suggest that invasive growth results in a disruption of polarity, as has been recently observed upon perturbation of growth in *Schizosaccharomyces pombe* (4). To further investigate cell morphology, as well as the distribution and dynamics of intracellular compartments, we are currently following a range of reporters, in particular during filament exit from the PDMS into a neighboring microchamber. These studies provide a framework for analyzing the interplay between mechanical constraints and polarity during invasive filamentous growth.

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OC-46

LINE-1 retrotransposition in murine and human muscle cells

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The human genome is formed by 47% of sequences derived from transposable elements. Long Intersperse Element-1 (LINE-1 or L1) forms the only currently active autonomous transposable element family in humans, and it is known to retrotranspose in human embryonic stem cells, in the germline, in epithelial tumors and in the brain.

There is no strong evidence to date that shows retrotransposition of L1 in other somatic tissues. In this study, we investigate the possibility of L1's mobility in the human and mouse muscle. We performed retrotransposition assays in both human and mouse myoblasts, to determine the permissivity of these cells to retrotransposition. Furthermore, we study the expression of L1's ORF1 protein in these cells, as well as in mouse and human muscle tissue samples. We found that myoblasts are permissive for L1 retrotransposition and that ORF1 seems to be expressed in mouse muscular tissue.

OC-47

Chronic telomeric stress impairs global organismal fitness and genomic stability in budding yeast

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At each cell cycle, conventional DNA replication fails to maintain chromosome ends resulting in chromosome shortening. To prevent this, the ribonucleoprotein telomerase adds nucleotides at telomeres maintaining their length constant, using a RNA moiety as template. Telomeric sequences are remarkably conserved in evolution and typically consist of repeated, short and G-rich units. Although most telomeric sequences share the same properties, some variation is present, especially between distant taxa. The nature of the repeated motif determines which accessory proteins are bound to the telomeres to distinguish them from double strand breaks (DSBs). When the repeat is modified, the proteins cannot recognize the telomere, resulting in the activation of the DNA damage response, that in turn leads to chronic telomeric stress. In the budding yeast S. cerevisiae, the telomerase RNA template is encoded by the gene TLC1 and synthetizes TG1-3 repeats. By artificially swapping a small fragment of the yeast RNA template with the human one, it is possible to generate a yeast strain with human-like telomeric repeats. In this work, we evolved multiple parallel lines of both humanized and wild-type yeasts for 2000 mitotic generations without selection in order to characterize the long-term effects of chronic telomeric stress. Yeasts with human telomeric repeats gradually slowed the mitotic cycle, had a shorter chronological lifespan and developed sensitivity to a broad array of conditions, including oxidative stress, heat shock and several anti-cancer drugs. Whole genome sequencing of the mutation accumulation lines revealed that humanized yeasts had a 2-fold increase in mutation rate and increased instability of the nuclear and mitochondrial genomes, which in turn led to an increased level of reactive oxygen species (ROS). Among the mutations isolated in humanized yeasts, two are found in TEL1/MEC1 genes. The humanized line carrying the mutation in TEL1 underwent catastrophic genomic rearrangements, including chromosomal translocations, subtelomeric amplifications and a whole genome duplication. However, this line showed an almost complete recovery of fitness compared to the other ones. Overall, our results revealed that dysfunctional telomeres might not only cause focal damage but have a global genome stability effect possibly mediated by the high levels of ROS.

OC-48

Overcoming genome complexity barriers in hybrids with meiotic reversion

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Meiosis is the biological process embedded in gametogenesis (sporulation in yeasts) that allow the halving of the genome content, and via recombination between the parental genomes, largely contribute to generate genetic and phenotypic diversity in the offspring. This process is evolutionary conserved among all eukaryotes, including single cell yeasts. However, gametes viability is impaired by multiple genetic factors (e.g. sequence divergence, gross chromosomal rearrangements, aneuploidies, gene incompatibilities) hence, limiting the possibility to apply classical breeding strategies. Here, we take advantage of a process called Return to Growth (RTG), in which yeast cells that enter meiosis are able to return to mitotic growth, and yield recombinant products without chromosomes segregation and ploidy reduction. Thus, reversion from meiosis, which occurs after the genome-wide initiation of meiotic recombination, can be used to generate recombinant cells, with potential phenotypic variation. The RTG process is induced by a shift in the culture media during the prophase of meiosis and result in genome-wide recombination leading to extensive loss of heterozygosity (LOH) regions. Here, we applied the RTG framework across a broad range of yeast diploid hybrids and investigated how the genomic architecture affect the recombination rates and landscapes. Moreover, we quantified the impact of the RTG in generating phenotypic diversity and exploited these recombinant libraries to map quantitative traits loci, opening the possibility to map traits in sterile hybrids, not feasible by means of classical linkage analysis.

OC-49

Altered gamma-secretase function leads to the enrichment of high molecular weight APP-CTFs in brain exosomes from Alzheimer mouse models

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Alzheimer's disease (AD) has been mainly associated with the aggregation of extracellular Ab and intraneuronal Tau. The Ab peptide is generated through sequential cleavages of the Amyloid Precursor Protein (APP). First it is cleaved by beta-secretase generating the membrane fragment C99, which then is cleaved by gamma-secretase leading to the production of the soluble Ab peptide. Recent work of my team demonstrated that AD mice not only accumulate extracellular Ab, but also display early and age-dependent endolysosomal accumulation of C99. Intriguingly, brains from these animals also present extracellular C99-associated immunostaining that was particularly strong in gamma-secretase inhibitor (D6) treated animals. The aim of this project was to characterize the nature of this extracellular staining and to determine the effect of gamma-secretase inhibition on the fate of C99.

Using in vitro (APPswe or C99-expressing SH-SY5Y or HEK cells) and in vivo (3xTg-AD mice or C99-expressing mice) AD models, we here demonstrate that this extracellular staining corresponds to exosome associated C99 (and C99-derived C83, generated by alpha-secretase cleavage), exosomes being small nanometer-sized vesicles originating from endosomes and released by cells. Indeed, exosomes purified from both brain tissue and cell culture media were found to contain high levels of both C99 and C83 (APP-CTFs). Interestingly, exosomes purified from D6 treated cells displayed increased exosomal C99 and C83, but also contained various high molecular weight (HMW) APP-CTFs, the latter being hardly detectable in whole cell or brain homogenates. Thanks to two C99 dimerization mutants (C99 G29L and C99 G29L/G33L), we established that these HMW APP-CTFs correspond to dimeric APP-CTFs including both homomeric (C99 and C83) and heteromeric (C83/C99) APP-CTFs. Furthermore, immunocytochemistry analysis revealed that, whereas monomeric C99 and C83 were localized to the trans-Golgi network and the endoplasmic reticulum, dimeric APP-CTFs were localized within endosomal and lysosomal compartments, thus explaining the selective recovery of dimeric APP-CTFs in exosomes.

Taken together, our study is the first to demonstrate the presence of exosomal APP-CTF dimers in mouse AD models and to show that dimerization is amplified upon gamma-secretase inhibition. Since exosomes are known to carry and spread pathogenic proteins our ongoing studies seek to evaluate the contribution of these APP-CTFs in AD progression.

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OC-50

The laterodorsal tegmental nucleus: a new actor in freezing

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Mammals are constantly challenged, both physically and psychologically, by environmental stimuli, which can persistently modify one's behaviors. These environmental stressors trigger a large panel of responses ranging from classical fight or flight responses to more complex modulations of cognitive processes. The stress response per se is adaptive and hence beneficial when promptly shut off. Studies in laboratory animals have commonly used foot shock exposure to study freezing behavior and associated fear responses. A wealth of evidence implicates the amygdala in the neuronal circuit underlying freezing or escaping behaviors. Here, we identified the laterodorsal tegmental nucleus (LDTg) as a new player in the circuit of stress-induced freezing. The LDTg is located in the brainstem and comprises intermingled cholinergic, glutamatergic and GABAergic neurons that send ascending projections to sensori-motor and associative brain regions. Using chemogenetic tools in transgenic mouse lines, we analyzed the contribution of the different LDTg cell types in response to mild foot shocks and monitored freezing behaviors as well as associated fear responses. We found that GABAergic, but not glutamatergic or cholinergic, neurons are key to modulate immediate freezing. Using ex-vivo patch recordings, we have measured the cellular changes induced by stress. Combining retrograde Cre-expressing viruses with Cre-dependent inhibitory DREADDs, we are currently dissecting the contribution of LDTg projections to these processes.

Our results establish a key role of the LDTg in freezing responses and further extend our knowledge of the neuronal circuitry behind the stress response, which could help for development of therapeutic intervention in the case of abnormal freezing behaviours.

OC-51

The role of Arf6 in Wg/Wnt signalling

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My project will address the role of Arf6 and its GEFs in the wingless pathway using genetic, functional assays and in vitro biochemistry. The Wnt/Wingless signaling pathway plays an essential role in embryonic development as well as stem cell maintenance and renewal, and its deregulation is implicated in a range of human cancers. The activation of the pathway is mediated by the binding of the secreted Wnt ligand to its receptor which in turn promotes the deactivation of the β-catenin destruction complex. This stabilizes cytoplasmic β-catenin which then accumulates in the cytoplasm and translocates to the nucleus where it acts as a transcriptional regulator for the activation of downstream targets.

Previous studies have demonstrated how the small GTPase Arf6 is rapidly activated downstream of the binding of Wnt to its receptor in melanoma cells. Following its activation, Arf6 has been suggested to act at two distinct levels in Wnt signalling, both in the assembly of the Wnt signalosome and the relocalisation of β-catenin. These results prompted us to look for a potential role of Arf6 in canonical Wingless signalling in vivo. Drosophila Arf6 mutants show a classical wingless phenotype in which the wing margins contains developmental defects. We are currently focussing on the identity of the guanine exchange factors necessary for the activation of Arf6 and how they are recruited by the wingless pathway. We are employing a range of genetic, biochemical and cellular approaches to thoroughly characterise the affinity, specificity, activity and localisation of the putative Drosophila exchange factors. Through this work we hope to understand at which level Arf6 acts in the Wnt pathway under physiological conditions using an in-vivo model.

OC-52

Evolution of developmental plasticity in the nematode C. elegans

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The dauer stage in C. elegans is a prime example of developmental plasticity whereby larvae adopt an alternative, stress-resistant larval stage in response to harsh environmental conditions. The molecular mechanisms regulating dauer induction have been well-characterized, however no study has determined the molecular basis explaining differences in dauer induction among natural C. elegans isolates. Here we characterized a C. elegans isolate (JU751, France), which shows an unusually strong propensity to form dauers at mildly stressful conditions compared to most other C. elegans isolates. Therefore, we performed a QTL analysis using F2 recombinant inbred lines. This analysis identified a single highly significant QTL on chromosome III, spanning approximately 750kbs. After further restricting the target region through fine-mapping, we focused on a single candidate variant: a 90bp deletion in the presumptive promoter region of eak-3, a gene known to be involved in dauer induction in response to high temperature. We present experimental evidence that this gene regulatory change is indeed the causal basis for the evolution of increased dauer induction in the JU751 isolate. In addition, we showed that the variant may provide a potential benefit to survive harsh environmental conditions.

OC-53

An optimal distribution of polyunsaturated acyl chains in phospholipids for fast membrane deformation and fission by endocytic proteins

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The team previously showed that polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins [1], suggesting a link between the striking enrichment of neuronal membranes in these lipids and the very fast speed at which endocytosis occurs in neurons [2]. To further explore the impact of polyunsaturated phospholipids on membrane deformation and fission, we perform experiments and simulations on membranes in which we control the distribution of polyunsaturated acyl chains in model membranes in two ways. First, we systematically change the acyl chain profiles of membranes by introducing an acyl chain of defined length and unsaturation level at position 1 and/or 2 of the phospholipids [3]. Second, we restrict the distribution of the polyunsaturated phospholipids in the inner or in the outer leaflet of the membrane. These approaches indicate that the combination of one saturated and one polyunsaturated acyl chain in phospholipids strikes a balance between impermeable and flexible membranes and that polyunsaturated phospholipids boost inward membrane deformation and fission when present in the inner (i.e. cytosolic) but not outer membrane leaflet. These two observations are in agreement with the distribution of polyunsaturated acyl chains in cellular membranes.

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OC-54

Protective mechanisms of pharmacologic preconditioning against myocardial ischemia reperfusion injury: role of Bcl-2 family proteins

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Myocardial ischemia reperfusion (IR) injury is the leading cause of perioperative morbi-mortality. Protective effect of pharmacologic preconditioning such as anesthetic preconditioning (APC) with sevoflurane (SEV) has been widely demonstrated in human and animal models. APC seems to protect myocardial cells from apoptosis, a programmed process of cell death tightly controlled by Bcl-2 family proteins. However, the involved mechanisms in APC have yet to be characterized. We hypothesized that APC protects against myocardial apoptotic cell death by regulating Bcl-2 anti-apoptotic members.

In order to study the SEV-induced APC mechanisms against IR, we used a validated in vitro model reproducing IR injury. Rat cardiomyoblast cells H9c2 were cultivated in 0.1% O2 hypoxia in the presence of ischemia-mimicking medium. After 90 mins of ischemia, reperfusion injuries are induced by replacing the culture medium with a Krebs-Henseleit pre-incubated normoxic medium in ambient air for 60 mins. APC was performed by adding SEV directly into the culture medium, prior to ischemia, for 60 mins. We then used another preconditioning agent, metformin (MET) to explore the same signaling pathways. Apoptotic cell death was measured by caspase activity assay and western blotting under IR and APC conditions.

Our model faithfully reproduced the protective effect of APC which results in a significant decreased apoptosis under IR (50% reduction of the Caspase 3 enzymatic activity, correlated with a decrease of Caspase 3 cleavage). We showed that SEV induces overexpression of the anti-apoptotic protein Bcl-xL, which is responsible for the protective effect of APC. Then, our observations were confirmed in vivo in mouse heart lysates. We demonstrated that Bcl-xL overexpression was due to the activation of the protein kinase Akt. Interestingly, we were able to show that preconditioning with MET reproduces the protective effect of SEV by inducing an Akt-dependent Bcl-xL overexpression.

Our results elucidates the molecular mechanisms by which SEV induces APC against IR injuries, i.e. the role of Bcl-xl (part of the Bcl2 family). Moreover, this study shows that pharmacologic preconditioning with the anti-diabetic drug MET promotes similar protective effect, sharing with SEV the same signaling pathways. Altogether, our results could be of interest to improve the perioperative management of patients at risk of ischemia reperfusion events, such as patients with a high cardiovascular risk.

Poster presentation
Abstracts

P-1

Low protein diet enhances tumor immunogenicity through activation of IRE1α

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Background. Reduction of the protein content of an isocaloric diet enhances a specific CD8+ T cell-dependent anti-tumoral response in immunocompetent mice. Such an effect of a low protein (low PROT) diet increased survival of melanoma and lymphoma mouse models and impaired tumor development of a colorectal carcinoma (CRC) syngeneic mouse model. Arresting of tumor growth by the low PROT diet was irrespective of either apoptosis induction or reduction in cell proliferation. The positive effect of the low PROT diet was indeed dependent on activation of the IRE1A pathway only in tumor cells leading to recruitment of cytotoxic T cells into the tumor microenvironment. IRE1A is an endoplasmic reticulum (ER) stress sensor and the trigger of one the branches of the Unfolded Protein Response (UPR) induced under ER stress.
Objectives. To decipher the molecular mechanisms by which a low PROT diet increases anti- cancer immunosurveillance and induces IRE1A activation within the tumor. In this regard, we have analyzed the surface expression levels of key immune markers known to modulate anti- cancer immune response in tumor cells of CRC tumor-bearing mice feed a low PROT diet. The differential expression of some immune markers on tumor cells suggests that low PROT diet modulates tumor immunogenicity making malignant cells more susceptible to cytotoxicity displayed by T cells. Whether this is linked to specific activation of IRE1A will be tested in immunocompetent mice. In addition, how low PROT diet modulates metabolism of CRC tumors leading to concomitant activation of the IRE1A pathway will be investigated by metabolomics analysis.
Perspectives. Altogether this PhD project will bring insights about the metabolic pathways that impact on tumor immunogenicity and might be targeted for enhancement of the immune response as the basis of future and novel anti-cancer therapies.

Key words. low PROT diet, IRE1A, UPR, ER stress, cytotoxic T cells, CRC, metabolomics

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P-2

Potentiation of immune checkpoint inhibitors by new anti-melanoma compounds

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Melanoma is one of the most lethal cancers with very bad prognosis due to its capability of invasion and metastasis. Even if encouraging results were obtained with BRAF and MEK inhibitors, these responses remain transitory and patients finally relaps (1). In parallel, therapies reactivating the immune system of the patient against the tumor (anti-PD1 and anti-CTLA4) were recently developed but give, at best, improved responses in maximum 30% of patients (2). Therefore, it's necessary to develop new therapeutic strategies for treatment of melanoma.

During the last few years, we have studied two families of drugs; the thiazole benzulfonadmides (TZB) and the biguanide families. They both have shown interesting anti-melanoma properties. Using structure/activity relationship in collaboration with the chemists from the ICN of Nice, we developed two lead compounds effectively promoting death of melanoma cells: HA15 (3) and CRO15 (article in preparation).
HA15, belonging to TZB family, has shown to bind to BIP (an endoplasmic reticulum protein) to trigger the Endoplasmic Reticulum (ER) stress which leads to cell death by the concomitant induction of autophagy and apoptosis. CRO15, derived from a biguanide, also provokes death by apoptosis and autophagy mainly by activation of the AMPK pathway. Interestingly, both ER stress and activation of AMPK pathway have shown to be involved in the anti-cancer immune response. While ER Stress has an important role in immunogenic cell death (4), the activation of AMPK pathway leads to increased infiltration of CD8+ T cells into the tumor (5).
Here, we showed that both HA15 and CRO15 reduce the tumor growth of melanoma allograft in an immunocompetent mice model. More importantly, both drugs potentiate the response to anti-PD1 treatment in a colon carcinoma allograft model improving thus the survival of mice and reducing drastically their tumor growth. Our initial in vitro and ex vivo results suggest that HA15 can reduce negative immune signals decreasing the production of cytokines such as IL10 and CCL2. On the other hand, CRO15 could increase the TH1 immune response via the secretion of IFN-γ.

Next, we would like to confirm the in vivo synergy of our drugs with anti-PD1 in a melanoma model and decipher more precisely immune mechanisms involved in this response.

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P-3

CD44 regulates NKp46+ Innate Lymphoid Cells behavior and their cross-talk with inflammatory macrophages during non alcoholic steatohepatitis

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Non alcoholic fatty liver diseases (NAFLD) are liver complications that develop with obesity. These complications are a major health issue with an increasing prevalence in western countries. The spectrum of these hepatic abnormalities extends from fatty liver to hepatic inflammation or steatohepatitis (NASH). NASH represents the progressive form of the disease and predisposes to more severe hepatic abnormalities such as fibrosis/cirrhosis or hepatocellular carcinoma. Despite lifestyle changes and bariatric surgery for severe/morbid obesity, the treatment of NAFLD (NASH) is still limited because of the lack of effective pharmacological treatment as well as lack of effective and practical diagnostic tools. Recent findings suggest that pathogenesis of NASH involves cross talks between different organs including liver, gut and immune system. The Innate lymphoid cells (ILCs) family regroups the well known natural killer cells (NK cells) as well as recently described resident epithelial helper-like cells (ILCs-1, 2 and 3). ILCs are main regulators of tissue homeostasis and inflammation and recent evidences indicate that ILCs populations regulate inflamed immune responses during obesity.

In the present study, we decided to focus on NKp46+ ILCs which include NK cells, helper-like ILCs-1/ILCs-3 as these subsets are major regulators of epithelial innate immune responses. In addition, our previous study demonstrates that the surface protein CD44, mainly expressed by immune cells is a key player in NASH by promoting macrophage and neutrophil infiltration which contributes to disease progression. In that context, we asked whether hepatic and gut NKp46+ ILCs participates to NAFLD development and whether CD44 affects NKp46+ ILCs physiology. To address these questions, we used a mouse model of steatohepatitis that reproduces the different stages of hepatic complications of obesity observed in humans. Here, we show that: i) CD44 is differentially expressed by NKp46+ ILCs subsets; ii) liver and gut NKp46+ ILCs subsets number are increased during NASH; iii) specific invalidation of CD44 on NKp46+ ILCs (NKp46+iCreCD44flox mice) aggravates liver injury and regulates liver inflammatory macrophages number in a mouse model of steatohepatitis. Altogether, our results highlight a key role of liver and gut NKp46+ ILCs in the regulation of hepatic inflammation and a regulatory role of CD44 in the dynamic of these ILCs subsets during NASH progression.

P-4

Myeloid cell glutaminolysis controls monocyte numbers and macrophage efferocytosis during atherosclerosis

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Background: Cardiovascular diseases (CVD) are a major public health issue in the modern society accounting for 17 million deaths per year. It is now known that monocyte count is an independent risk factor predicting disease progression and severity, and represents an attractive therapeutic

opportunity. Glutamine plasma concentration is associated with increased monocyte count and increased risk of cardiovascular diseases, but the underlying mechanisms remain unknown.

Hypothesis: We hypothesized that monocyte glutaminolysis modulation may contribute to atherosclerosis development.

Methods and Results: We generated a myeloid cell specific deletion of the rate-limiting enzyme hydrolyzing glutamine into Glutamate, called Glutaminase 1 (Gls1), in APOE deficient mice. These mice had increased number of peripheral blood monocytes associated with increased plaque area as compared to control mice. Mechanistically, these mice demonstrated accelerated monocyte recruitment to plaque although in situ macrophage proliferation in plaque was not affected. Transcriptomic and functional analyses revealed defective efferocytosis in Gls1 deficient macrophages.

Conclusion: We identified myeloid cell glutaminolysis as a critical player in CVD development. A better understanding of the metabolic pathways affected by Gls1 loss could provide new therapeutic targets to control myeloid cell functions during atherosclerosis.

P-5

Understanding asparagine synthetase heterogeneity and its impact in the metabolism of B-cell lymphomas

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Diffuse Large B-Cell Lymphoma (DLBCL) is the most common and aggressive type of Non-Hodgkin B-Cell Lymphoma (NHBL) that is characterized by high metabolic heterogeneity. The Eμ-Myc transgenic mice, a highly heterogeneous model of NHBLs, has been validated by our team for the study of DLBCL metabolic heterogeneity. We identified that Eμ-Myc lymphomas express heterogeneous levels of asparagine synthetase (ASNS), the enzyme involved in de novo asparagine biosynthesis. Such ASNS heterogeneity is maintained in vitro in Eμ-Myc -derived cells and we therefore classified Eμ-Myc lymphomas as ASNShigh and ASNSlow. We next hypothesized that ASNSlow lymphomas may be dependent on extracellular asparagine to sustain tumor growth. Indeed, both L-asparaginase treatment and asparagine removal in vitro have a negative impact on viability and proliferation of Eμ-Myc-ASNSlow cells. In addition, ASNS protein levels correlate with mRNA levels, indicating a different transcriptional regulation between ASNShigh and ASNSlow lymphomas. Interestingly, we found that high ASNS levels correlate with protein expression of the tumor suppressor P53 in vitro, suggesting that P53 status could regulate ASNS expression in Eμ-Myc lymphomas. Asparagine bioavailability is known to play an important role in the metabolism of cancer cells but the consequences of distinct ASNS expression levels on metabolic pathways remains unknown. In order to understand the impact of ASNS in the metabolism of Eμ-Myc lymphomas we performed steady-state metabolomics to find differentially expressed metabolites between Eμ-Myc -ASNShigh versus ASNSlow cells. Finally, to further analyze which metabolic pathways are specifically activated in Eμ-Myc-ASNShigh versus ASNSlow lymphomas we will trace glucose and glutamine carbon sources by isotope tracing

metabolomics. Altogether, this project will give new insights on the impact of ASNS expression and asparagine bioavailability in the development of Eμ-Myc lymphomas, as well as uncover potentially related metabolic vulnerabilities that could be exploited as a therapeutic option for DLBCL patients not responding to current treatments.

P-6

Role of mechanotransduction in melanoma cell plasticity and progression

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INTRODUCTION : Cutaneous melanoma is a very aggressive skin cancer due to its high metastatic potential and its resistance to treatments. Treatment of metastatic melanoma was improved in 10 years with the development of targeted therapies and immunotherapies. However, resistance occurs quickly in patients and they relapse. Studies of the team focused on tumor microenvironment. Tumor is an complex environment in which tumor cells is in contact with healthy cells and matrix proteins. This contact helps cancer progression. One of the main functions of the microenvironment is to provide an extracellular matrix that produces biochemical and mechanical signals sensed by cancer cells that will help their dissemination and protect them against treatments. However, the composition and the influence of these signals on melanoma progression are not well known. The goal of my thesis is to study how biochemical and mechanical signals of the extracellular matrix influence melanoma progression.

MATERIAL : For my project, I use in vitro models of 3D extracellular matrix derived from healthy fibroblasts or cancer associated fibroblasts and also polyacrylamide gels with increased stiffness to mimic biomechanical signals of melanoma microenvironment.

RESULTS : My results suggest that the microenvironment is modified during melanoma progression, with an better organization of the extracellular matrix fibers in the tumor. In addition, I show that melanoma cells can feel changes in the microenvironment stiffness by the activation of mechanotransduction pathways such as YAP/TAZ and MRTF/SRF signaling pathways. At the functional level, it is associated with an increase in melanoma cell proliferation and invasion properties when they are plated on stiff substrate.

CONCLUSION : These results suggest that melanoma cells are able to feel mechanical signals of their microenvironment and use them for their progression. My thesis project should allow a better understanding of the process that help melanoma metastatic progression and identify new signaling pathways involved in the link between melanoma cell and its microenvironment.

P-7

Adipose tissue derived fatty acids control monocyte mobilization

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Monocytes are mononuclear phagocytes generated in the bone marrow compartment. The pool of bone marrow monocytes depends on chemokine gradients that control monocyte mobilization to peripheral blood vessels or their retention inside the bone marrow. CXCR4-CXCL12 interaction is responsible for monocyte retention in the bone marrow, whereas CCR2-CCL2 interaction favors monocyte egress into the blood. In peripheral blood two subsets of monocytes are found: Ly6Clo (patrolling) monocytes are healing the endothelium and phagocyting debris, and Ly6Chi (inflammatory) monocytes can enter into peripheral tissues and differentiate into macrophages. However, the metabolic clues involved in monocyte mobilization remain ill-defined.

We aim to decipher the mechanisms implicated in the metabolic control of monocyte pool. We focused on fatty acids metabolism and generated deficient mice for adipose tissue lipolysis (AdipoQcre/ERT2 x Atglfl/fl). Using this genetic model, we found a significant decrease in Ly6Clo and Ly6Chi monocyte number and frequency in blood and spleen in AdipoQ cre/ERT2 x Atglfl/fl in comparison to littermate control animals. We failed to detect an impact on monocyte counts in the bone marrow compartment in AdipoQEr2cre x Atglfl/fl mice. Blood monocyte reduction was paralleled by an increase of CXCL12 protein in serum while CCL2 was not affected. Currently, we investigate the mechanisms leading to monocyte reduction by analyzing monocyte proliferation, death and retention.

P-8

Exercise and PPARβ agonist treatment improve immunometabolic and aerobic capacities in the context of diet-induced weight loss in obese female mice

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Obesity promotes the installation of a low-grade systemic inflammation, a defect in oxidolipidic metabolism and a progressive loss of skeletal muscle function.

Metabolism and immunity are tightly integrated in their respective effects: inflammation pathways are involved in metabolism and metabolism plays a predominant role on immune cells development and function. Inhibition of glycolysis in M1 macrophages or in Th17 cells directs them to their anti-inflammatory phenotypes cells. Briefly, pro-inflammatory immune cells preferentially depend on glycolytic metabolism, while anti-inflammatory cells of oxidolipid metabolism (Berod et al., 2014).

Exercise and dietary changes are recommended as non-drug therapies for the obesity care (Gleeson et al., 2011), these strategies may have possible impact on nutrients availability that could alter function and migration of immune cells. The consideration of these strategies in the obesity care is unclear. A therapeutic approach resulting in increasing PPARβ activity would favor an anti-inflammatory immune phenotype. Indeed, PPARβ not only promotes the oxidolipidic metabolism in both at the cellular level, for example in T cell (Mothe-Satney et al., 2016) and within the organism as a whole, but regulates AMPK activity which is crucial regulator of development and function of anti-inflammatory Treg cells (O'Neil et al., 2016). If non-drug strategy effects may be beneficial in the context of obesity-induced inflammation but it still remains unclear mechanism and if PPARβ agonist improves this classical strategy (diet, exercise) for obesity associated-risks reduction.

Could this effect promote an anti-inflammatory phenotype, beneficial in the context of obesity? We conducted a study in diet-induced obese mice subjected to 8-wk normal diet. In this context, we investigate the effects of 8-wk PPARβ agonist treatment combined or not with exercise. Alone, diet is not sufficient for normalization of visceral adipose tissue mass and systemic inflammation. Our results suggest a metabolic reprogramming of lymphoid organs with PPARβ agonist potentiated by exercise. These effects are associated with an increase in percentage of Treg cells, an improvement insulin sensitivity, a decrease inflammation and VAT mass, and an increase in oxidative capacities. We conclude that activating the PPARβ pathway, as a therapeutic approach, would improve or prevent obesity-associated diseases in reinforcing the recommended non-drug measures.

Keywords : Immunometabolism, obesity, PPARβ, physical activity, nutrition

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P-9

Turning intestinal somatostatin+ cells into beta-like cells

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Over the past few decades, diabetes has become one of the most widespread metabolic disorders with an epidemic dimension affecting almost 9% of the world's population. Diabetes can be subdivided into two main conditions: type 1 diabetes, which is mainly caused by an autoimmune-mediated destruction of pancreatic insulin-producing beta-cells and type 2 diabetes, resulting from the resistance to insulin action in peripheral tissues and eventual beta-cell failure. Both forms of diabetes result in chronic hyperglycemia which, without treatment, could lead to dramatic consequences, including vascular complication, blindness, amputation, or even death. Importantly, despite the most recent advances in diabetes care, patients suffering from diabetes still display, on average, a

shortened life expectancy and a worsened quality of life as compared to healthy individuals. There is therefore a need for alternative therapies.

Towards this goal, our laboratory focuses its researches on understanding the molecular mechanisms involved in endocrine cell neogenesis with the ultimate goal of forcing pancreatic beta-cell (re)generation as an alternative therapy for diabetes. Thus, using the mouse as a model, we could demonstrate that pancreatic glucagon-producing or somatostatin-producing cells could be turned into beta-like cells by misexpressing Pax4, a key transcription factor for beta-cell fate. Recently, we focused on endocrine cells in the gastrointestinal tract in vivo and we could provide evidences suggesting that intestinal somatostatin-cells could also be turned into insulin-producing cells upon Pax4 misexpression. In addition, we are developing organoid-based approaches to determine whether such intestinal insulin-producing cells are functional with the final goal of applying this methodology to human cells.

P-10

The role of the transcription factor E2F1 in pancreatic alpha/beta-cell identity and plasticity.

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According to The World Health Organization, diabetes represents one of the most common disease which is continuously rising. Diabetes is a chronic metabolic disease that can be divided into Type 1 Diabetes, Type 2 diabetes, gestational diabetes and Maturity Onset Diabetes of the Young (MODY).

The main focus of our lab is in Type 1 diabetes: it results from an early onset autoimmune disorder characterized by a chronic hyperglycaemia due to the progressive loss of pancreatic insulin-producing beta-cells. In order to develop new therapeutic strategies to improve the quality of life of diabetic patients, it is important to investigate the molecular mechanisms underlying the development and functionality of beta cells.

It has thus become clear in recent years that cell cycling proteins play a key role in the control of the metabolism, among which E2F1. E2F1 is a transcriptional factor involved in cell cycle progression and apoptosis, plus it has been shown to play a direct role in the glucose homeostasis. Accordingly, in vitro results demonstrated a loss of the beta cell identity upon E2F1 ablation. In order to determine whether a similar outcome could be obtain in vivo, we are generating transgenic mice allowing the constitutive or inducible loss of E2F1 in beta-cells. In addition, we are also generating animals permitting the misexpression of E2F1 in alpha-cells to monitor their outcome. Combining immunohistochemistry with functional studies, preliminary results suggest that the mice misexpressing E2F1 in alpha-cells display an improved response to glucose stimulation. Further investigations are ongoing to determine whether a conversion of alpha-cells into beta-like cells is occurring. Together, this work should allow us to determine whether the modulation of E2F1 function could be beneficial for diabetic patients.

P-11

Opposite effects of Neuropilin-1 and Neuropilin-2 in the aggressiveness of clear cell Renal Cell Carcinoma

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Anti-angiogenic therapies targeting VEGF-A or its receptors, are used for the treatment of metastatic clear cell renal cell carcinoma (ccRCC). The current reference therapy in the first line is the multi-kinase inhibitor sunitinib (Sutent®). However, relapses appear after a few months. We recently described that VEGF-C, the main pro-lymphangiogenic factor, represents one of the main actors of a progressive disease with dissemination of tumour cells through the neo-formed VEGF-C-dependent lymphatic network. These results highlight the urgent need to develop alternative therapeutic strategies for ccRCC at relapse on conventional treatment.

A distinct family of VEGFs co-receptors, the Neuropilins (NRPs) form complexes with VEGFs and their receptors and induce tumour cell proliferation, migration, and survival, favour angiogenesis and immune cell exhaustion resulting in tumour growth and dissemination. NRP-1 and NRP-2 were associated to a poor prognosis in several cancer types, which has led to the development of pharmacologic inhibitors of their activity. However, the involvement of NRP in the aggressiveness of ccRCC was poorly investigated. By inhibiting the expression of NRP-1 or NRP-2 by shRNA, we showed that the NRP-1/VEGF-A pathway favoured proliferation and migration whereas the NRP-2/VEGF-C pathway inhibited proliferation and migration of ccRCC cells. We developed a pharmacological inhibitors of both NRPs, NRP-a308, that presented anti-tumour effects on triple negative breast cancer models only expressing NRP-1. NRP-a308 inhibited the proliferation and the migration of ccRCC cells expressing both NRPs more efficiently than sunitinib. However, NRP-a308 did not inhibited the growth of experimental ccRCC in nude mice. The opposite roles of NRP-1 and NRP-2 pathways may explain this result. Indeed, administration of NRP inhibitors should be considered with caution either only in patients whose tumour only express NRP-1 or through the design of highly specific NRP-1 inhibitors."

P-12

The phosphatidylserine flippase Drs2 has a unique role during Candida albicans invasive growth

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Phospholipid flippases (P4-ATPases) transport lipids across the membrane bilayer to generate and maintain membrane asymmetry; these ATPases translocate lipids from the external leaflet to the cytosolic leaflet of cellular membranes. There are 5 flippases in Candida albicans, including Drs2, which we showed to be critical for hyphal invasive growth (1). Indeed, a drs2 deletion mutant grew similar

to the wild-type strain during budding growth, yet was deficient for invasive growth and, in particular, unable to maintain hyphal growth. This mutant had an altered distribution of phosphatidylserine (PS), as assessed with a fluorescent reporter, and was additionally hypersensitive to fluconazole. Very recently, this flippase was also shown to be critical for copper sensitivity (2). To delineate the role of Drs2 during C. albicans hyphal growth, we investigated the dynamics of distribution of this flippase, as well as that of different lipids and key regulators, during initiation and maintenance of hyphal growth. We also characterized a Drs2 point mutant, analogous to that shown to be altered for PS flipping in S. cerevisiae (3). Furthermore, we examined the role of other flippases, such as Dnf2, in invasive hyphal growth, together with their importance in response to membrane stress. All together, our results indicate that Drs2 has a unique role during C. albicans hyphal growth maintenance, which appears to be particularly critical upon septum formation.

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P-13

Impact of Vascular PPARbeta/delta Expression on Tumor Progression and Metastasis Formation

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Neovascularisation is one of the crucial steps in the transition of a tumor from a small cluster of malignant cells to a visible macroscopic tumor capable of spreading to other organs via the vasculature. Thus, angiogenesis is considered as one important hallmark of cancer. Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors, which belong to the nuclear receptor superfamily including PPARalpha, PPAR beta/delta, and PPAR gamma. PPARbeta/delta has been suggested to be involved in the regulation of the angiogenic switch in tumor progression. However, it is still unclear to what extent the expression of PPARbeta/delta in the tumor endothelium influences tumor progression and metastasis formation. Thus, it is very interesting to study its potential function in tumor endothelium. For this purpose, we generated a double-transgenic mouse model with an inducible conditional vascular endothelial cell-specific PPARbeta/delta overexpression after Tamoxifen injection.

We did subcutaneous implantation of lung carcinoma cells and measured tumor growth and subsequently embedded samples in paraffin for histological analysis. Quantitive RT-PCRs and RNA-sequencing were performed to identify the potential PPARbeta targets, and then we confirmed these by molecular biological technologies including transient transfection, cloning and ChIP-assays. We observed enhanced tumor progression and tumor metastasis formation, and higher vessel density in mice with endothelial PPARbeta overexpression compared to controls. Interestingly, we identified Pdgfrb and Pdgfb as new direct targets of PPARbeta, which was confirmed in ChIP assays. We can conclude that selective overexpression of PPARbeta in endothelial cells leads to faster tumor growth and larger tumor sizes; and metastsis formation is enhanced. Therefore, the use of PPARbeta agonists for the treatment of metabolic syndrome seems to be critical, but it might be dangerous to boost

endurance of athletes. In contrast, PPARbeta/delta inhibition might become a novel approach in tumor therapy.

Key words: PPARbeta/delta overexpression, angiogenesis, tumor progression, metastasis formation and ChIP-assays.

P-14

Newly infiltrated neutrophils under CpG + aIL10Ra peritumoral treatment play a key role in antitumor immunity and tumor regression

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Skin carcinomas represent one of the most common cancer in the world. Current treatment strategies, surgery and radiotherapy are not very effective against invasive forms. The establishment of a treatment involving a long-term immune response would control its invasive forms and recurrences. To stimulate innate and adaptive immunity we developed an immunotherapy based on the peritumoral injection of a Toll Like Receptor agonist TLR9L together with an antibody blocking the signaling through the IL-10 receptor. This therapeutic strategy induces the total regression of established medium-size skin tumors in a mouse model. The total regression of tumor is associated with a massive recruitment of neutrophils at the site of the tumor. Newly recruited neutrophils are highly activated and produce large amounts of ROS. This work will help to understand the molecular mechanisms involved in the functions of tumor-associated innate immune cells and ultimately will optimize a local and self-administrable strategy of immunostimulation against cutaneous carcinomas.

P-15

Genetic disruption of the cystine importer xCT (SLC7A11) reduces growth, survival and tumorigenicity and increases sensitivity to chemotherapy of pdac cells

(Capan-2 AND MiaPaCa-2)

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The system xCT acts as a Na+-independent and Cl--dependent antiporter of the anionic forms of cystine (oxidized form of cysteine) and glutamate. The activity of this system is crucial for the maintenance of the cellular redox balance as the uptake of cystine is required for the synthesis of intracellular glutathione (GSH). GSH represents the major antioxidant molecule of the cell, also involved in biotransformation of xenobiotics and essential in protein folding. Recent pharmacological studies suggested xCT as potential target for redox-based anti-cancer therapy. In order to further

investigate this issue, particularly in pancreatic ductal adenocarcinoma (PDAC) we made a knockout of the xCT transporting subunit in Capan-2 and MiaPaCa-2 cell lines using the CRISPR-Cas9 technique. These cells were then characterized for GSH content, proliferation, clonogenicity, and survival. Considering that cysteine-to-cystine in blood is subjected to changes, focus of the study was also placed on the importance of the xCT for these cells to make tumors in vivo.

Both xCT-KO cell lines had markedly reduced GSH content, leading to an increased accumulation of oxidative stress marker - lipid peroxides, which ultimately resulted in massive cell death by ferroptosis. As expected, cultivating xCT-KO cells under reducing conditions (N-acetyl-cysteine or ?-mercaptoethanol) that allow bypassing the activity of xCT, restored cellular GSH content, proliferation and survival of xCT-KO cells. Also, accumulation of lipid peroxides and survival of the mutant cells were prevented by two inhibitors of ferroptosis: Vitamine E or an iron chelator - deferoxamine (DFO). Present study showed that pharmacological inhibition of xCT by a low concentration of erastin (1?M) phenocopies its genetic disruption, and increases susceptibility of PDAC cells to chemotherapeutics such as gemcitabine or cisplatine.

Furthermore this study showed for the first time that genetic disruption of xCT negatively affects tumorigenic potential of both cell lines. However, the cells were still able to slowly develop tumours in vivo suggesting two possibilities: 1) residence mechanisms are involved in adaption xCT-KO cells to in vivo environment and/or 2) cysteine-to-cystine balance in the blood permits adaptation and survival of xCT-KO in mice by potentiating cysteine transport.

P-16

Non-small cell lung cancer infiltrating immune cells express a truncated P2RX7 splice variant impairing the proper localization of P2RX7 to the cell membrane

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Introduction: P2RX7 is an extracellular-ATP cation channel receptor made of three monomers. Its activation increases Ca2+ and large cation (macropore) influxes and ultimately leads to cell death. P2RX7 was described to be over expressed in tumor tissues but also to play an important role in anti-tumor immune responses. The goal of this study was to understand how P2RX7, which triggers cell death, could be overexpressed in tumors. We hypothesized that expression of P2RX7's splice variants may impair its functional activity. Indeed, 12 alternative splice variants were described for P2RX7, some of which leading to the loss of functional domains.

Methods: We designed specific probes to quantify the expression of P2RX7 splice variants by real time PCR. These probes were used on human NSCLC in tumor and non-tumor areas in both immune and non-immune cells. The biological activity of P2RX7 was tested by flow cytometry, using Topro3 dye to assess the macropore formation and fluo4-AM to assess Ca2+ influx. We used the bi-molecular complementation approach and confocal microscopy to document the hetero-oligomerization of P2RX7.

Results: In NSCLC, we detected the expression of P2RX7-A (full-length) and 3 alternative splice variants namely, P2RX7-B, P2RX7-H, P2RX7-J. In both tumor and non-tumor tissues P2RX7-A and -B are expressed, and their level of expression is decreased in the tumor area. P2RX7-B lacks the C-terminal extremity which is involved in proper localization of the receptor in cell membrane. By contrast, P2RX7-H, P2RX7-J are expressed at a very low level in the lung tissue. In addition, we showed that, whatever the expression level of splice variants is, P2RX7 is not functional in non-immune cells (epithelial and stroma) purified from both tumor and nontumor areas. Further, we demonstrated that the expression of P2RX7-B retains heterotrimeric P2RX7-A/B within the cytoplasm and consequently affects the biological function of the receptor. Finally, we showed that P2RX7 is less functional in immune cells purified from the tumor versus non-tumor area. This loss of function correlates with higher expression level of P2RX7-B in these particular cells.

Conclusions: P2RX7 is not functional on tumor cells of NSCLC. In addition, patients with tumor showed less P2RX7 activity in tumor immune cells. These cells expressed P2RX7-B, suggesting that this splice variant could affect the efficiency of antitumor immune response.

P-17

Multimodal and Multidimensional assessment of apathy

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Apathy is defined as a reduction in goal-directed activity compared to the previous state of functioning in the domains of behaviour/cognition, emotions and/or social interactions.
These changes can be reported by the patient himself or through external observation. This disorder may be present in several neuropsychiatric disorders (depression, schizophrenia) or neurocognitive disorders (AD, Parkinson's, DFT).
Apathy is essentially assessed through clinical scales. This type of evaluation may not be very objective, with the risk that patients or even clinicians may be influenced by the context or their moods when the scales are completed either by the clinician himself, the patients or the caregiver without reflecting the reality.
The use of nTICs for objective assessment of motivation disorders using the MoTAP (Motivation Application) application. Method: MoTAP is part of the MNC3 program (Digital Medicine Cognition Behavioural Brain) at the University of the Côte d'Azur. This application aims to evaluate, through behavioural tasks (in the form of games) and sensors (audio, video, actimetric) the different dimensions of apathy.
Behavioural tasks are designed to assess goal-oriented behaviours and cognitions, and reward sensitivity. Audio and video sensors can record patients' voices and faces respectively to highlight the presence of emotional blunting during emotionally induced tasks using voice and facial characteristics Actimetric sensors are used to assess the participants' activity level when performing behavioural tasks. A first prototype of MoTAP was tested at the Memory, Resources and Research Centre of the University Hospital of Nice in 60 subjects of average age 74 years (+/- 8.3) with an average Mini Mental State (MMSE) score of 23 (+/- 5.2).

Résultats : Preliminary results suggest that the MoTap set up is able to differentiate apathetic from non-apathetic patients in terms of behavioural task data and facial state. Speech and facial analysis did not show any results for this protocol, but showed some promising results in the current set-up. Conclusion: These preliminary results confirm the value of using ICTs to improve the objective assessment and quantification of apathy in neurocognitive and neuropsychiatric disorders.

P-18

Transposable Elements activity and role in Meloidogyne incognita genome dynamic and adaptability.

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Root-knot nematodes (genus Meloidogyne) are among the most damaging plant pathogens with a worldwide economic impact estimated at several billions of euros per year. Within this genus, the mitotic parthenogenetic species M. incognita is one of the most successful parasites. Despite being unable to combine beneficial mutations from different individuals and purge deleterious mutations via recombination, this species is able to adapt to different conditions and break down resistance by the host plant. Therefore, the paradoxical polyphagy and adaptability of M. incognita, despite the absence of sexual reproduction, must rely on mechanisms generating genetic plasticity. Our group previously sequenced the genome of M. incognita and discovered that it has an allopolyploid structure (triploid hybrid) (1).

Transposable Elements (TE) are known to provide plasticity in genome sequence and their role can be particularly important in hybrids.Hence, we investigated the potential of TE as a mechanism to generate the necessary plasticity for adaptability in M. incognita.

We first performed a comprehensive de novo detection and annotation of TE in the genome of M. incognita. We found that ~25.5 % of the genome is repetitive. Repetitive elements that could be assigned to Class I or Class II span ~19% of the total genome size (185 Mb). We identified known TE-related protein (reverse-transcriptase, transposase, integrase, etc) in 11.4% of the TE, suggesting they could be active.

We have sequenced at high coverage, pools of individuals from 11 different M. incognita geographical isolates, covering 4 different pathotypes and 5 different crops of economic importance (soybean, coffee, cotton, watermelon, cucumber) (2). We used these pool-seq data to trace TE activity and mobility across the 11 M. incognita isolates. We found 171 insertion loci for which TE presence is heterogeneous between isolates, a good indicator that the concerned TEs were active recently.

We also found that all of these loci are close (< 1.5 kb) or inside genes. This suggests TE activity at these loci could have a functional impact by disrupting or modifying gene expression and might be related to phenotypic diversity among M. incognita isolates.

These preliminary results set the basis for further analyses to assess whether TE plays an active part in M. incognita ability to adapt to its environment despite its lack of sexual reproduction.

Blanc-Mathieu et al. PLOS genetics. 2017.

P-19

A two-tier junctional machinery under the control of embryo cross-patterning drives coordinated tissue folding and elongation

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Morphogenesis is a process by which the embryo is reshaped into the final form of a developed animal. Tissue morphogenesis is under the control of genes which expression follows precise and instructive patterns that can extend from the anterior to the posterior (AP) or from the dorsal to the ventral (DV) axis of the embryo. While much work has been done in understanding how AP and DV patterning independently control morphogenesis, little is known on how cross-patterning functions. We use the Drosophila embryo as a model system and focus on the process of tissue folding, a process that is vital for the animal since folding defects can impair neurulation in vertebrates and gastrulation in all animals which are organized into the three germ layers. Past work has shown that an acto-myosin meshwork spanning the apical-medial side of prospective mesoderm cells and under the control of the embryo DV patterning plays a key role in mesoderm invagination. Nevertheless, experimental evidence and theoretical simulations have argued that apical constriction per se is not sufficient for invagination. In our lab, we have uncovered a cell junctional lateral network under the control of both AP and DV patterning. This contractile network generates tension along the apical-basal axis and within the tissue plane 10-15 µm inside the mesoderm epithelium initiating lateral cell intercalation. Lateral forces in mesoderm cells seem to play a multivalent role both driving mesoderm extension and invagination. Finally, by implementing 4D multi-view light sheet imaging, infra-red femtosecond ablation to perturb the cytoskeleton and optogenetics to synthetically control tissue morphology, this work shines new light on the origin and functions of a novel mechanism responsible for coordinated tissue elongation and folding.

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P-20

Organoids of intermediate mesoderm derived tissues from mouse ES cells

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The intermediate mesoderm (IM) represents an important developmental structure that gives rise to kidneys, gonads and the adrenal cortex. Recent advances have allowed the generation of kidney organoids from human Pluripotent Stem Cells (hPSC) or Induced hPSC (IhPSC) that contain most of the cell types found in the adult kidney. However, human stem cells differ from mouse embryonic stem cells (mESC) and a reliable protocol for the generation of kidney organoids from mESCs is still missing. In addition, differentiation of stem cells into adrenal and gonadal organoids has not been reported.

Our lab is interested in IM-derived organs and the signaling pathways that induce cells to commit to kidneys, adrenals and gonads. Here we describe the differentiation of mouse ES cells into IM that involves a first induction step towards the EpiSC (epiblast derived stem cells) state followed by Wnt activator (CHIR99021), FGF and activinA treatment. Subsequent induction with FGF9 allowed differentiation into posterior IM that expressed markers specific for nephron progenitors (eRPCs). When cultured in 3D and treated with CHIR, eRPCs can be further induced to undergo mesenchyme-to-epithelial transition (a key step in nephrogenesis) and to form renal organoids that are composed of all important nephron components.

In a second line of investigation, we are testing pathways that permit differentiation into adreno-gonadal progenitor cells. Once established, this protocol will allow us to address key events in adrenal development and tissue homeostasis and may in the long run help to devise regenerative approaches for adrenal diseases. More generally, employing mouse ES cells established from genetically modified mice (e.g. Cre-lox strains), will be a powerful tool to dissect the molecular mechanisms driving the development and differentiation of IM mesoderm derived organs in vitro.

P-21

Identification of active module in an interaction graph using node2vec network embedding

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IBV, I3s

We propose a computational strategy for aid in the identification of cancer-activated module based on gene expression data from Pancreatic ductal adenocarcinoma (PDCA) samples. A cancer-activated module is a set of genes that interact with each other to control cellular malfunctions inder cancer progression. Discovering genes that have relations to this type of genetic alteration can help to identify triggers that produce a great effect of deregulation in the gene network and that are directly related to cancer.

P-22

Effects of the interaction between chronic stress and Otx2 expression on the habenula-interpeduncular functions

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The habenula-interpeduncular system (HIPS) is a key neuronal circuit involved in social and reward-related behaviors. It is composed of the medial habenula (MHb) and the interpeduncular nucleus (IPN), both expressing the Otx2 transcription factor from developmental stages. Several studies have shown that a perturbation of this circuit, in adulthood, leads to psychiatric disorders such as anxiety and depression. However, although psychiatric disorders affect mainly adults, they may arise from an

alteration of neuronal circuits occurring during critical periods of development in which these circuits are more sensitive to the impact of the environment and the expression of developmental genes. So far, this aspect has been poorly studied in the HIPS. Here, we are investigating the effects chronic stress combined with a modulation of the expression of Otx2 in the HIPS, during its critical period of development, on the onset of psychiatric disorders. Our preliminary data suggests that in mice, the HIPS is more sensitive to the effects of stress around the post-natal days P37-P43. Next, we will study, in two different ways, the effects of Otx2 expression modulation in the HIPS at the critical period, on the development of anxiety and depression. First, we will induce an Otx2 conditional knock-out in the MHb combined with a chronic stress during the critical period. Second, we will transiently knock-down Otx2 in the IPN combined with a chronic stress during the critical period. These results will allow us to identify the effects of Otx2 expression modulation on the susceptibility to develop psychiatric disorders.

P-23

Role of AMPK in mitochondrial dysfunctions and neuroinflammation in Alzheimer disease

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Mitochondria dysfunction (i.e. structure and function alterations) is one of the earliest features in the brains of Alzheimer's disease (AD) patients [1]. In this pathology, in addition to extracellular A? senile plaques, APP (Amyloid Precursor Protein)-derived proteolytic fragments interfere with different cellular pathways participating to the pathological features of AD [2]. The C-terminal fragment (CTF)? (C99) was shown to accumulate early in a transgenic mice model mimicking AD and to be toxic when over-expressed [3]. We and other laboratories reported that APP and its derived fragments (C99 and A?) are present in the microdomain between the endoplasmic reticulum and mitochondria, [4], controlling mitochondria structure and function [5]. Recent studies have revealed that one ancestral function of Adenosine Monophosphate-activated Protein Kinase (AMPK) is to promote mitochondrial health, and multiple newly discovered targets of AMPK are involved in various aspects of mitochondrial homeostasis, including the elimination of damaged mitochondria (mitophagy). Moreover, a recent work underlines the involvement of AMPK in inflammatory response [6].

The aim of my PhD work is to elucidate the involvement of the AMPK molecular cascade in these mitochondrial dysfunctions, C99 accumulation and in the activation of neuroinflammatory cells.

We first shown mitochondrial accumulation of C99 in human-derived brains as demonstrated by sub cellular fractionation and Western Blots. This was further confirmed by immunohistochemistry analyses. Similarly, accumulation of C99 in mitochondria was observed in two AD study mice models (the triple transgenic (3xTg-AD) and AAV-10 expressing C99 models).

We used the human neuroblastoma cells overexpressing or not APP carrying the Swedish familial mutation (APPswe), known to overproduce APP catabolites (i.e. C99 and A?). We investigated the

AMPK signaling cascade and the impact of its pharmacological modulation on: i) APP processing and C99 accumulation in mitochondria; ii) Mitophagy; and iii) Mitochondrial functions.

My future studies aim to confirm the pharmacological modulation results in the two AD mice models, and to also modulate the AMPK with a genetic approach (lentiviruses expressing WT, super active and dominant negative AMPKs). Besides the mitophagic state, mitochondrial functions and APP-CTFs accumulation, these in vivo models will be used to examine the involvement of AMPK in neuroinflammation.

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P-24

Lysophosphatidylcholine modulates ASIC channels and induces long-lasting joint pain in mice in an ASIC3-dependant manner

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Pain is a main trigger for a patient to visit a physician. Those with chronic joint pain, represent a large population seeking medical care and pain relief. Because chronic joint pain pathophysiology is poorly understood, mainly due to a lack of specific molecular targets, analgesic treatments prescribed today are disappointing. Improving patient care by discovering new therapeutical targets is a major health concern, all the more knowing today's scourge of painkiller misuses and abuses.

Ion channels, such as ASICs (« Acid-Sensing Ion Channels »), have emerged as important candidates in nociception mechanisms. Expressed in the central and peripheral nervous system, they play a key role in nociception signaling. Until recently, ASICs channels were known to be activated only by extracellular acidosis. Interestingly, a lipid found in human synovial fluids from patients suffering from chronic joint pain, lysophosphatidylcholine (LPC), has been shown to strongly modulate ASIC3 channel activity.

Here, we report that LPC not only activates ASIC3 at physiological pH (7.4), without any extracellular acidification, but also potentiates ASIC3 current amplitudes in response to extracellular acidosis. LPC doesn't induce a direct activation at pH7.4 of ASIC1a, but current amplitudes in response to extracellular acidosis were potentiated.

ASICs are trimeric ion channels made of different subunits (ASIC1, 2 & 3). A wide range of arguments suggest that ASIC isoforms mostly combine as heterotrimers in vivo, the ASIC3+ASIC1 heteromers being predominant in sensory neurons. In ASIC3+ASIC1a transfected cells, LPC effects were

comparable to ASIC1a transfected cells: no direct activation at pH7.4, but potentiation of acid-induced currents.

We also show in vivo that LPC intra-articular injections produce chronic pain in WT mice, lasting for at least a month. Interestingly, ASIC3 knock-out mice appear to be protected from this LPC-induced chronic pain state.

These data demonstrate that ASICs are positively modulated by LPC, a lysolipid found at high level in human patients suffering from painful joint pathologies. ASIC3 is particularly sensitive to LPC, with both activating and potentiating effects. Importantly, this work reports that LPC is able to trigger an ASIC3-dependant chronic pain state when injected in mice joints.

Focusing on ASIC3 and its regulators, such as LPC, may enlighten chronic joint pain pathophysiology, and could offer new strategies to improve patient care.

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P-25

Characterization of a synaptic SUMO2/3-ylome.

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SUMOylation is a very dynamic post-translational modification that regulates the activity, stability, subcellular localization and protein-protein interactions of its target proteins. Originally characterized as a nuclear protein modification, SUMOylation emerged as a key regulator of extranuclear functions. An increasing number of neuronal proteins have been shown to be sumoylated and evidence for alterations in SUMOylation were reported in many neurological diseases. It's now clear that the SUMOylation is essential for the regulation of several neuronal processes like neuronal excitability[1-3], post-synaptic differentiation [4] as well as in synaptic transmission and plasticity[5-7]. A deeper understanding of the role of SUMOylation in the brain now requires a global screen of sumoylated proteins. Using a specific SUMO2/3 antibody, we performed immunoprecipitation and peptide elution on synaptosomes to isolate endogenous SUMO2/3-ylated synaptic proteins. By mass spectrometry approaches, we identified around 800 sumoylated proteins at the synapses. To validate the MS list, I employed immunoprecipitation and SUMO blot for 4 different targets. Our data provide invaluable resources to globally assess the regulation of synaptic functions by SUMOylation. Interestingly, some of our top candidate are associated to neuronal diseases. Therefore, identifying sumoylated proteins known to be involved in neurological disorders open up new perspectives to understand the link between the SUMOylation and pathologies.

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P-26

On the export mechanism by RND proteins: AcrB's structure-based study

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RND family proteins are transmembrane proteins identified as large spectrum drug transporters [1]. The paradigm model of those proteins is AcrB, a protein found in bacteria. It was identified as responsible for antibiotic resistance in selected gram negative bacteria, but the drug efflux mechanism is still under debate.

AcrB forms an homotrimer, and the available structures are either symmetric ones (all subunits in the same state), or asymmetric ones (subunits are in three different states, ABE). In its asymmetric state, the protein extrudes a drug molecule against proton uptake. Unfortunately, the size of the system (1049 amino-acid per monomer and membrane) is such that dynamic simulations failed to unveil the detailed mechanism [2].

This study [3] makes a stride towards a finer understanding of the export mechanism, exploiting the known crystal structures (35) as well as novel modeling tools.

First, we show that all asymmetric trimers occupy the ABE state.

Second, we exhibit states for domains of AcrB, and ascribe these to states of whole subunits.

Third, we characterize the conformational changes undergone by the domains of a given subunit, which is key to correctly classify monomers and trimers.

Fourth, we delineate the evolution of contacts between subunits and the drug during the export mechanism.

Finally we confront the obtained results to the rest of RND's available structures.

Altogether, these insights pave the way to performing dynamic simulations of AcrB, by focusing on those degree of freedom which are key during the export mechanism. In turn, these findings may foster the design of molecules blocking the efflux.

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P-27

An electrostatic switching mechanism controls the activity of PS / PI4P exchanger

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A central assumption is that lipid transfer proteins (LTPs) bind sequentially and transiently to organelle membranes to distribute lipids in eukaryotic cell. In yeast, Osh6p and Osh7p are LTPs that transfer phosphatidylserine (PS) from the endoplasmic reticulum (ER) to the plasma membrane (PM) via PS/phosphatidylinositol-4-phosphate (PI4P) exchange cycles. Yet it is unknown how, at each cycle, they escape from the electrostatic attraction of the highly anionic PM to return to the ER. Using cellular and in vitro approaches, we show that Osh6p dissociates from anionic membranes once it captures PS or PI4P, due to a molecular lid that closes its lipid binding pocket. Osh6p thus maintains a fast transport activity between ER- and PM-like membranes. Computational and experimental investigations reveal that the lid governs the membrane docking and activity of Osh6p because it contains an acidic motif. Jointly, our data unveil how an LTP uses an electrostatic switching mechanism to self-limit its residency time on membranes in order to be efficient

P-28

Gut microbial metabolite contributes to the development of autistic-like behaviours in mice

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Autism spectrum disorder (ASD) is a frequent neurodevelopmental disorder (1:67) characterized by impairment in social interaction, communication deficits, as well as by repetitive behaviours and restricted interests. ASD is also frequently accompanied with anxiety and hyperactivity and gastrointestinal tract dysfunctions, including microbiota dysbiosis. Recent studies demonstrate the existence of a gut-brain axis through which the intestinal microbiota is able to impact behaviour. We hypothesised that metabolites produced by the gut flora could mediate these behavioural effects. We focussed on p-Cresol, an aromatic microbial metabolite, notably produced by bacterial metabolism of tyrosine in the human intestine by Clostridium difficile. This metabolite was previously shown as upregulated in urine of autistic children, in agreement with reports of overabundance of Clostridium

spp. in the microbiota of at least subtypes of ASD patients. In addition, the abundance of p-Cresol was correlated with intensity of stereotypies and intestinal disturbance in ASD patients.

We studied the intestinal and behavioural consequences of chronic p-Cresol treatment in C57BL/6 mice. We show that p-Cresol treatment elevated urinary excretion of p-Cresol and dramatically increased paracellular and transcellular permeability, while it reduced faecal output in mice. These data are compatible with the symptoms of constipation and the “leaky gut” syndrome described in at least subtypes of ASD patients. In addition, animals treated with p-Cresol display an increase in perseverative behaviours and motor stereotypies as well as strong impairments in social interactions, which are core behavioural symptoms of ASD. The behavioural effects of p-Cresol treatment are extremely selective as other dimensions of behaviour (anxiety, locomotor activity) remain unaffected. Our study suggests that bacterial metabolites may convey information from the gut microbiota to the brain and could be directly involved in the development and maintenance of autistic behaviours, through disruption of the intestinal barrier. Furthermore, it underlines that restoring intestinal homeostasis is a possible therapeutic avenue for ASD.

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P-29

Targeting Phosphodiesterase 2A in the Fragile X Syndrome

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Fragile X syndrome (FXS) is a rare genetic neuro-developmental disorder with a prevalence of 1:4000 males and 1:7000 females. It represents the most common form of inherited intellectual disability and a leading cause of autism spectrum disorder (ASD). FXS originates from the loss of the expression of the FMR1 gene which encodes FMRP, an RNA-binding protein that modulates the translation of a subset of synaptic proteins. While no specific therapy for this disorder is yet available, current treatments focus on behavioral therapy and off-label medications that mitigate a limited set of symptoms, such as hyperactivity, seizure and anxiety.

By using HITS-CLIP(Cross-Linking ImmunoPrecipitation) we found that the mRNA coding Phosphodiesterase 2A (Pde2A) is the most prominent target of FMRP in 13-day-old mouse brain, especially in the cortex and in the hippocampus. This age is in the temporal window where FMRP is most highly expressed in the mouse forebrain and the synaptogenesis reaches a peak in cortex and in hippocampus. PDE2A is mainly a synaptic enzyme involved in cGMP-dependent degradation of both cAMP and cGMP exerting both pre- and post-synaptic functions. cAMP and cGMP are second messengers at the crossroad of numerous signaling pathways that modulate memory and cognition.

In FXS the level and activity of PDE2A are increased and, consequently, the abundance of both cAMP and cGMP is reduced.

Blockade of PDE2A, by using its specific inhibitor BAY607550, restores the normal length of growing axon (that is reduced in FXS) and improves the morphology of dendritic spines in Fmr1-null primary cultured neurons that appear immature. Furthermore, the inhibition of PDE2Arescues the exaggerated mGluR-dependent LTD in hippocampal slice that is a hallmark of Fmr1-null brain.

Moreover, the acute injection of BAY rescues the deficit of social interaction and vocalization in newborn and adolescent Fmr1-null mice. While I will present here new data showing the chronic treatment of FXS by Bay607550, the future project will be extended to the characterisation of various phenotypes of the Fmr1-null mouse after genetic reduction of Pde2a.

P-30

Therapeutic strategies for mitochondrial diseases using cellular and murine model.

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Within the past few years, the team studied two different families presenting with typical mitochondrial myopathy where some patients also harboured symptoms of Fronto-Temporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) (Bannwarth and Saadi et al., 2014). These two neurodegenerative diseases are commonly due to multiple factors but in these cases, we have been able to determine that a missense mutation (c.176C>T) was at the origin of the symptoms. This mutation leads to a change of a Proline into a Leucine (p.S59L) in a previously unstudied protein : CHCHD10. Since, we have shown that CHCHD10 is a mitochondrial protein enriched in the intermembrane space, more specifically at the cristae junction. CHCHD10 is thought to interact with the mitochondrial contact site and cristae organizing system (MICOS complex) whose role is to maintain mitochondrial cristae structure. We have shown that the mutation p.S59L leads to the disorganization of MICOS complex, provoking a loss of mitochondrial cristae, a diminution of the respiratory chain activity, a loss of mitochondrial DNA and an inhibition of cell death by an inhibition of the release of cytochrome c (Genin et al., 2016). We continued our work by generating and analysing a new Knock-In mouse model carrying the mutation p.S59L (Genin et al., 2019). As FTD-ALS is a redoubtable disease and because several teams showed that different point mutations in the CHCHD10 gene were found in cases of Charcot-Marie-Tooth type II disease and several other neurodegenerative diseases (Penttilä et al., 2015; Auranen et al., 2015), we aim to discover new therapeutic strategies for neurodegenerative diseases linked to a disorganization of the MICOS complex. For that, we decided to look for molecules approved by the Food and Drug Administration (FDA) that could have a beneficial impact on the symptoms due to MICOS disassembly. Our goal is to procced to a therapeutic repositioning. We focused on two molecule banks (Prestwick and SelleckCHEM) and we use the different models to our disposition as filters to find molecules that would have a beneficial effect. We already passed through a first filter: a mutant yeast (S.cerevisiae) for the MICOS complex. Now, we are using different patient fibroblasts carrying the mutation p.S59L

and we will next use motor neuron derived from patient IPSCs. We hope to finally be able to test those molecules in our mouse model.

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P-31

Study of the regulation of telomerase activities by a phosphatase called pX.

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Telomerase serves two independent and critical roles in normal stem cells and in cancer. As a reverse transcriptase that synthesizes telomere repeats, telomerase maintains telomere length and stability to prevent the severe adverse consequences of telomere dysfunction, which include senescence, apoptosis and massive chromosome instability. In order to add telomere sequences to chromosome ends, telomerase requires both TERT, the telomerase protein component, and TERC, the telomerase RNA component.

In addition to this well documented telomerase activity, recent studies demonstrated that TERT possess a non-canonical activity, that facilitates proliferation of quiescent epidermal stem cells in a murine conditional TERT expression model. Moreover, it has been shown that TERT causes a dramatic effect on kidney epithelium, resulting in acute cell cycle entry and loss of differentiation markers.

Despite the importance of TERT activities in stem cells and in cancer, much remains to be understood regarding TERT protein regulation. Although telomerase is regulated in part at the level of TERT transcription, there is evidence that the protein is tightly regulated post-transcriptionally. However, very few data are available regarding TERT phosphorylation dynamic and its biological significance.

The aim of my PhD is to determine if and how a phosphatase called pX regulates telomerase activities. To address this question I used in vitro approaches to assess the impact of pX on the ability of telomerase to elongate telomere sequences. In addition, I used sophisticated mouse genetic approaches to determine if pX modulates TERT non-canonical functions on kidney epithelium. My preliminary results suggest that pX is necessary for deployment of TERT non-canonical functions, and that pX restrains telomerase canonical activity on telomere. Those results suggest that pX plays a pivotal role in regulating the balance between canonical and non-canonical functions of telomerase. I will further determine in the upcoming months how pX modulates telomerase functions at the molecular level.

P-32

Role of the telomeric protein TRF2 in post-mitotic cells.

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Cells exposed to a stress (mitotic, oxidative, oncogenic…) can become senescent, a stable arrest state of the cell coupled to phenotypic changes(1). And it is admitted that accumulation of senescent cells leads to aging of the organism. But some cells, notably long-lived post mitotic cells (LLPMC, like muscle cells or neurons), do not become senescent, but they age through their lifespan and this led to a progressive decline of the organism fitness. Indeed, aged LLPMCs experience an increase of ROS, a decrease of autophagy and an altered proteostasis(2-3). However, the link between these age-dependent cellular changes and the "classical" cellular senescence pathways are still unclear.

Until now the only known cellular aging "clock" is telomere shortening. Telomeres are composed of tandem repeat arrays containing the TTAGGG motif. To preserve genome stability, telomeric DNA is associated with shelterin, a protein complex composed of TRF1, TRF2, RAP1, TIN2, TPP1 and POT1(4). It has been observed that telomeres shorten in aged LLPMC as well as the levels of TRF2 decreased(5).

We previously showed the ability of TRF2 to regulate gene expression with a predominance of neuronal genes(6-7).

Is the TRF2-dependent expression responsible of the functional LLPMCs decrease during aging?

To answer this question, we are deciphering how TRF2 can regulate gene expression. We first hypothesized that Interstitial Telomeric Sequences (ITS, repetition of TTAGGG motif located in intergenic regions) could exert a TRF2-dependent enhancer activity. Using a candidate approach, we are trying to see how TRF2 can regulate PPP2R2C, an important brain specific subunit of a phosphatase. We generated cells in which we deleted a candidate ITS by CRISPR-Cas9 to see how PPP2R2C regulation by TRF2 is affected and decipher by which mechanisms.

We are also using a genome-wide approach by looking at chromatin landscape changes induced by TRF2 using ChIP-seq.

We are also exploring another physiological role of TRF2. Using a strong microfluidic device, allowing us to observe axonal transport in mouse neuron primary cultures, we found that TRF2 regulates axonal transport. Using different mutants of TRF2 we are now trying to understand which domain of TRF2 is necessary for this new role.

This work would allow us to reveal new pathways and to improve our understanding on the link between telomeres, LLPMC function and aging. It would bring, as well, new insights to corroborate the program aging.

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P-33

XRN1: a protein at the crossroad between pbodies and cytoplasm

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Lung cancer is the leading cause of cancer death in France and worldwide, due to its high rate of recurrence. These relapses are the consequence of onset of cells resistant to anticancer treatments. One hallmark of cancer cells is to adapt very quickly to new hostile environment and survive. However, the mechanisms of early resistance to anticancer agents is poorly known. In the laboratory, we hypothesized that storage of RNAs in "processing bodies" is involved in very early resistance of cancer cells. Indeed, these pbodies appears to quickly sequester dispensable mRNA (15 to 30min) during different cellular stress (change of temperature, deprivation in glucose ...) to improve survival with resting fraction of mRNA. However, the precise role of pbodies has never been studied in a cancer context.

To examine whether induction or inhibition of pbodies were associated with resistance, I choose to focus on XRN1 protein, a well-known pbodies protein. Indeed, XRN1 seems to be at the crossroad between RNA degradation or storage in the cell since XRN1 is located in pbodies while this activity is cytoplasmic. The goal of my thesis is to determine i/how and why XRN1 protein interacts with pbodies, ii/what are the proteins that target XRN1 to pbodies, and iii/ how XRN1 is involved in anticancer drug resistance.

To answer these aims, I generated cell lines invalidated for XRN1 expression to study the RNA decay in absence of XRN1. Furthermore, I made constructs to study specific domains involved in pbodies accumulation.

My results confirmed that XRN1 is in charge of 5’ to 3’ decay in cytoplasm. Furthermore, I also showed that the Cterm of the protein XRN1 is responsible for pbodies targeting. More precisely it seems that 2 domains within the Cterm are necessary for this addressing. Now I’m trying to determine the proteins associated to these domains.

Altogether, we want to determine how XRN1 is regulated and whether its regulation is at the onset of the cancer resistance.

P-34

FOXO3a role for telomeres homeostasis and skeletal muscle aging.

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Telomeres are composed by short repeated DNA sequences (TTAGGG) at the end of chromosomes. They ensure genomic stability and they prevent chromatids fusions and DNA damage machinery to recognize the end of chromosomes as double stranded breaks. However, telomeric DNA shortens at each round of cell's division. Indeed, telomere shortening is one of the most known drivers of aging because it drives replicative senescence. Although well described in mitotic cells, telomere importance in aging of post-mitotic cells like muscle fibers remains elusive. Previous studies in post-mitotic tissue, uncovered that the level of TRF2, a key telomeric protein, declines in aging muscle without inducing telomere damage and senescence. Instead, this TRF2 downregulation leads to ROS accumulation, mitochondrial dysfunction and FOXO3A translocation into the nucleus. Amazingly, the FOXO3A relocate to telomeres, where they act as protective factors to compensate for the telomere-uncapping effect of TRF2 diminution. Therefore, telomere protection in aged muscle cells relies directly on FOXO3A activity, revealing an unexpected link between the FOXO longevity pathway and telomere protection in long-lived post-mitotic cells. Here, we want to characterize FOXO3a binding at telomeres in mitotic and post-mitotic cells. We look forward to elucidate the reason triggering FOXO3a protection, how does it bind to telomeres and which is it role in telomere homeostasis.

P-35

The temporal varibility of migration can decrease the expected genetic differencition of sub-divided populations

Flora AUBREE

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Spatial isolation between sub-populations of the same species is one of the principal sources of genetic differentiation and a possible cause of speciation. When such sub-populations remain partially connected, the resulting gene flow tends to homogenize the two gene pools. Existing theory mostly consider migration as a constant exchange rate between populations. However, migration is often governed by non constant phenomena that can be periodic or completely stochastic, and therefore gene flow is likely to be highly temporally variable. Some theoretical arguments have suggested that the more variable the migration rate, the higher the expected genetic divergence, but few studies have addressed this question, and were restricted to neutral genetic variation. In this work we study the consequences of a pulsed gene flow pattern for the genetic divergence of two sub-populations. We model episodic events of genetic exchange, and how they interact with genetic drift, mutation and selection to determine the genetic divergence rate.

Four genetic scenarios are considered: (i) neutral divergence ; (ii) divergent selection where populations specialize on each habitat and migrants are mal-adapted (local adaptation) ; (iii) accumulation of deleterious recessive mutations (buildup of inbreeding depression) ; and (iv) hybrid incompatibility (accumulation of confronting it with stochastic Monte-Carlo simulations. By quantifying the probability of genetic identity between the two populations, we find that depending on the genetic scenario and other parameters, genetic divergence may be higher or lower with pulsed migration than with continuous migration.

(1) Whitlock et al. Temporal Fluctuations in Demographic Parameters and the Genetic Variance among Populations. Evolution, 1992(2) Carlton et al. Tsunami-driven rafting : Transoceanic species dispersal and implications for marine biogeography. Science, 2017

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