Journées de l'Ecole Doctorale de Nice

2018

24th and 25th May 2018

Faculty of Science, Valrose, Nice

abstract book

Keynote Speakers

Matthias Landgraf

University of Cambridge, UK

Petra Bleeker

University of Amsterdam and Enza Zaden, Netherlands

ÉCOLE DOCTORALE 85 Sciences de la Vie et de la Santé

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ÉCOLE DOCTORALE 85
Sciences de la Vie et de la Santé

Detailed Program

Thursday, May 24th

8h00 – 8h45 Registration/breakfast

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

9h00 – 9h45 Invited Keynote Lecture: **Matthias Langraf**: Department of Zoology, University of Cambridge – United Kinkdom

9h45 – 10h50 Oral Communication Session 1

OC-1 **Nadia Formicola**
From RNPs to memory: Imp and CamKII in activity-dependent remodelling.

OC-2 **Sara Castagnola**
A targeted pharmacological approach for Voltage Gated Calcium Channels highlights the role of Cav2.1 in calcium flux deregulations in Fragile X neurons.

OC-3 **Sarah Zerimech**
Hyperactivation of GABAergic neurons and cortical spreading depression: a new pathological mechanism of migraine.

OC-4 **Renaud Bussiere**
Post-translational-mediated Ryanodine Receptors calcium leak leads to Alzheimer's disease-like pathologies, and cognitive deficits.

OC-5 **Wejdane El Manaa**
Study of UPR function in Parkinson's disease.

OC-6 **Torsten Felske**
Direct reprogramming of cells into corticospinal motor neurons.

10h50 – 11h20 Coffee Break

11h20 – 12h40 Oral Communication Session 2

OC-7 **Perrine Royal**
Alternative translation initiation as a molecular mechanism of K2P potassium channel dysfunction in Migraine.

OC-8 **Anaëlle Grabek**
Sexual diphormism of the adrenal cortex is driven by hormone dependent activation of distinct stem cell compartments.

OC-9 **Furong Tang**
Rspo1 in Female Sex Determination.

OC-10 **Nainoa Richardson**

Analysis of Sox8 and Sox9 in XX R-spondin1 Sex Reversal.

OC-11 Morgane Le Rolle
Determine the role of the WNT/β-catenin signalling pathway in germ cell differentiation.

OC-12 Vishnu Saraswathy
Analysing the role of the E3-ubiquitin ligase Mindbomb1 in planar cell polarity signalling.

OC-13 Jonas Friard
Role of LRRC8/VRAC in Epithelial-to-Mesenchymal Transition (EMT).

12h40 – 13h10 Lunch Break

13h10 – 14h30 Poster session 1 (from **P-1** to **P-25**)

14h30 – 15h15 Invited Keynote Lecture:

15h15 – 16h20 Oral Communication Session 3

OC-14 Katharina Stobbe
CCL5 protects against the development of obesity, diabetes and associated neuropathic pain.

OC-15 Gwenaëlle Le Menn
Effect of T cell specific PPAR? overexpression on diet-induced obesity and its associated inflammation and insulin resistance.

OC-16 Xi Yao
Brown-like adipocyte progenitors derived from human iPS cells: A new tool for anti-obesity drug discovery and cell based therapy.

OC-17 Martin Paré
Deciphering the adipose tissue browning in physiopathological contexts.

OC-18 Estelle Roger
Microrna involvement in the control of brown adipose tissue activity in a model of predisposition to type 2 diabetes.

OC-19 Gaia Fabris
Possible role of miRNAs in the adaptation of hepatocytes to amino acid deprivation.

16h20 – 16h50 Coffee Break

16h50 – 18h00 Oral communication Session 4

OC-20 Alexandre Bourgeois
Amyloid-beta independent implication of the C99 fragment in Alzheimer's disease: study of a new Alzheimer's mouse model based on viral expression.

OC-21 Charles-Vivien Olivieri
Epstein-Barr Virus infection in gingival tissues: towards new paradigm in oral pathogenesis.

OC-22 Aidan Falvey

Micro-organ discovered to be a potential electroceutical for inflammatory disorders.

OC-23 **Lucile Hurault**
Effect of natural uranium on osteocyte mineralisation function and autophagy.

OC-24 **Raphaël Bonche**
Role of Perlecan in BMP signalling pathway.

OC-25 **Amélie Cavard**
Active Sonic Hedgehog pathway is required for the airway multiciliated cell differentiation.

18h00 – 19h00 Drinks

19h00 – 21h30 Dinner

Friday, May 25th

8h30 – 9h Registration

9h00 – 9h45 Invited Keynote Lecture: **Petra Bleeker:** Enza Zaden & University of Amsterdam – Netherlands

9h45 – 10h50 Oral Communication Session 5

OC-26 **Cyril Van Ghelder**
The Ma gene for resistance to root-knot nematodes: insights into its unique features among TIR-NBS-LRRs.

OC-27 **Danila Cabral Do Nascimento**
WEE1 checkpoint control is necessary for a proper Root-Knot Nematode feeding site development.

OC-28 **Van Chung Nguyen**
Phylogeography of the vector nematode *Xiphinema* index using mitochondrial and microsatellite markers highlights its Eastern origin closely linked to grapevine domestication.

OC-29 **Yusha Wang**
Conservation biological control: Impact of the various non-cropped components of landscape elements on phytophagous and natural enemies.

OC-30 **Yanyan Qu**
Impact of pest functional types on plant-mediated indirect interactions.

OC-31 **Victor Burte**
Effect of density and host distribution on the spatial diffusion of *Trichogramma cacoeciae*.

10h50 – 11h20 Coffee Break

11h20 – 12h40 Oral Communication Session 6

OC-32 **Marjorie Haond**
The Range Pinning: When populations are frozen in space.

OC-33 **Luo Chen**
The presence of secondary symbionts modulates phenoloxidase (PO) in the pea aphid and the host susceptibility to biotic and abiotic stress.

OC-34 **Claire Michelet**
Phylogenetic study of Macrophage Migration Inhibitory Factor (MIF) cytokines and analysis of their role in host-parasite interactions

OC-35 **Adrien Paul**
Cell composition of periodontal ligament extemporaneously just after dental extraction and after culture

OC-36 **Anthony Ruberto**
The role of KLF10 in the circadian coordination of liver homeostasis

OC-37 **Pierre Eric Danin**
Noninvasive evaluation of non-alcoholic fatty liver disease by assessing liver function and serum markers

OC-38 **Karine Dumas**
REDD1 deficiency protects from high fat diet induced metabolic diseases

12h40 – 13h10 Lunch Break

13h10 – 14h30 Poster Session 2 (from **P-26** to **P-50**)

14h30 – 15h40 Oral communication Session 7

OC-39 **Joana Justino**
PLA2R1 as the major autoantigen in Membranous Nephropathy: from epitope spreading to personalized medicine.

OC-40 **Christelle Zaghrini**
Novel ELISA for Thombospondin type 1 domain-containing 7A autoantibodies in membranous nephropathy.

OC-41 **Vladimir Kozlov**
Analysis of SOX11 function in early and late mouse kidney development.

OC-42 **Ly Ta Ngoc**
The tyrosine phosphorylated prosurvival form of Fas intensifies the EGF-induced signal in colorectal cancer cells through the nuclear EGFR/STAT3-medicated pathway.

OC-43 **Hala Awina**
Dissection of the role of the E3 ubiquitin ligase LNX2 in the control of Fas Cell death signalling potential applications in preventing colorectal cancer recurrence.

OC-44 **Lisa Kaminski**

PGC-1a controls an onco-metabolic program to limit prostate cancer aggressiveness.

15h40 – 16h20 Coffee Break

16h20 – 17h30 Oral Communication Session 8

OC-45 Charlotte Cohen
Fractalkine (FKNs) within a multi-modal therapy protocol of NSCLC bone metastasis: pre-clinical study in a murine model.

OC-46 Ruxanda Moschoi
Chemotherapeutic stress triggers a protective horizontal mitochondrial transfer from mesenchymal stromal cells to AML leukemic blasts.

OC-47 Racha Fayad
Loss of EFA6-B, an EMT regulator, facilitates breast cancer development in vivo.

OC-48 Ilona Berestjuk
Discoidin Domain Receptors DDR1 and DDR2 promote matrix-mediated drug resistance (MM-DR) to MAPK-targeting therapies in BRAF mutated melanoma.

OC-49 Déborah Vallée
Inhibition of the IRE1? Branch enhances the anti-tumoral activity of Sorafenib in hepatocellular carcinoma.

OC-50 Sanya Kuzet
Matrix stiffness contributes to the chemoresistance o head and neck squamous cell carcinoma to EGFR inhibitors

OC-51 Justine Leclerc
Role and regulation of ASAH1 in melanoma.

18h00 Closing session and JEDNs award

Poster Index

Session 1 (Thursday, May 24st)

P-1 **Camille Syska**
The nitrogen-fixing symbiosis : role of bacterial VapBC toxin antitoxin modules.

P-2 **Julia Matonti**
Involvement of Kir2.1 in the BMP pathway maintenance during osteoblastogenesis.

P-3 **Pablo Ávalos Prado**
Deciphering molecular and cellular pathways in pain control: nature as inspiration for the rational design of new potent analgesics.

P-4 **Rana Mhaidly**
GAPDH overexpression in the T cell lineage promotes angioimmunoblastic T cell lymphoma through an NF-B dependent mechanism GAPDH overexpression in the T cell lineage promotes angioimmunoblastic T cell lymphoma through an NF-B dependent mechanism.

P-5 **Marion Tiberti**
Impact of phospholipid polyunsaturation and asymmetry on membranes as deduced from molecular dynamics simulations.

P-6 **Joffrey Mejias**
A root-knot nematode effector targets the spliceosomal plant machinery facilitating the giant cells formation.

P-7 **Li Yang**
Regulation of senescence-specific proteases during the nitrogen-fixing symbiosis in *Medicago truncatula*.

P-8 **Tatiana Gritsaenko**
Characterization of bone extracellular matrix produced by RECQL4-deficient osteoblast.

P-9 **Malgorzata Drozd**
Functional characterization of new genes implicated in Early Onset Schizophrenia and Autism.

P-10 **Laïla Giordano**
Characterization of Individual Domains of the Arabidopsis Receptor-Like Kinase IOS1.

P-11 **Henri Montaudié**

P-12 **Marta Garcia Prieto**
Impact of the FXS-linked R138Q mutation on FMRP sumoylation and function.

P-13 **Monserrat Vazquez Rojas**
The role of EFA6B in epithelial morphogenesis and its implication in breast cancer progression.

P-14 **Marwa Zerhouni**

P-15 **Kavya Vinayan Pushpalata**
Novel Insights Into Neuronal RNP Granules Upon Aging.

P-16 **Clémence Canivet**
The hepatokine FNDC5/Irisin is a protective factor against Non-Alcoholic Fatty Liver.

P-17 **Romain Rozier**
Protective mechanisms of anaesthetic preconditionning with halogeneted agents against myocardial ischemia reperfusion injury: role of Bcl-2 family proteins.

P-18 **Gaurav Pandharikar**
Cross-talk between aphid facultative symbiosis and plant nitrogen fixation symbiosis in the *Acyrthosiphon pisum* - *Medicago truncatula* interaction.

P-19 **Morgane Plutino**
Targeted next generation sequencing with an extended gene panel does not impact variant detection in mitochondrial diseases.

P-20 **Alice Rouan**
Telomere Dynamics among Pacific Corals.

P-21 **Melania D'Angiolo**
Genome instability initiate introgressions in budding yeast.

P-22 **Sandra Dhifallah**
Rescue of a novel familial hemiplegic migraine folding defective NaV1.1 mutant leads to gain of function: common mechanism?

P-23 **Sarah Fazio**
Investigating the role of Ro60/sY-RNAs complex in lipid-laden macrophages and adipocytes.

P-24 **Pierre Leclère**
Role of the Circadian Transcription Factor KLF10 in the development of Non Alcoholic Fatty Liver Diseases.

P-25 **Iris Grosjean**
GENEXPOSOMICS Linking genetic to environmental factors for prevention of lung cancer.

Session 2 (Friday, May 25st)

P-26 **Pasquale Pensieri**
Otx2: a neuroprotective signal for Adult Retina Photoreceptors?

P-27 **Serena Silvano**
RSPO1: a key player for proper endocrine cell function in the pancreas.

P-28 **Lucilla Fabbri**
VDAC1 and ciliopathy.

P-29 **Audrey Valverde**
The impact of various N- and C-truncated A? peptides in Alzheimer's disease.

P-30 **Cecilia Colson**
Control of brite adipogenesis by fatty acid metabolites.

P-31 **Marine Gautier**
Functional characterization of long non-coding RNAs modulated by hypoxia in Non-Small Cell Lung Carcinomas.

P-32 **Rouba Jneid**
Btk toxins influence progenitor cell fate of intestinal stem cell.

P-33 **Serena Diazzi**
The pro-fibrotic miR-143/145 cluster regulates an extracellular matrix remodelling program during adaptive and acquired resistance of melanoma cells to targeted therapies.

P-34 **Charles Puerner**
Organelle Dynamics During Fungal Filamentous Growth.

P-35 **Bérengère Dadone**
The FGF/FGFR signaling pathway: a novel therapeutic target in liposarcoma.

P-36 **Paula Peressini**
With a little help from my friend: a nested LINE-1-Sleeping Beauty system to measure LINE-1 retrotransposition from chromosomal positions.

P-37 **Anaïs Bécot**
Modulation of Lysosomal-Autophagic Pathway in Alzheimer's Disease.

P-38 **Maria Mensch**

Unravelling the physiopathological actions of the newly discovered A? peptides in the brain.

P-39 Marie Deprez
Study Tracking cell trajectories by single-cell transcriptome analysis during airway epithelium regeneration.

P-40 Fabien Muselli

P-41 Vivien Weber
The impact of intronic LINE-1 insertions on host gene expression.

P-42 Julien Marcetteau
Understanding the role of Arf6 in the wingless signaling pathway.

P-43 Simone Mozzachiodi
Overcoming genome complexity barriers in hybrids with meiotic-reversion.

P-44 Charlotte Pandiani
Deciphering the molecular mechanisms involved in senescence of choroidal melanocytes.

P-45 Christopher Rovera
Factors secreted by invasive melanoma cell lines inhibit the contraction of lymph node fibroblasts.

P-46 Loïc Broussot
Parsing the role of LDTg projections in stress-related disorders.

P-47 Cristina Paraschivescu
The role of the chemokine CCL17 in brain development and behavior.

P-48 Giorgia Miloro
Regulation ot T cell activation by Fas receptor.

P-49 Meng-Chen Tsai
Polyunsaturated phospholipids facilitate the closure of transendothelial cell macroaperture induced by bacterial toxin.

P-50 Mickaël Meyer
The Response heterogeneity of clonal cancer cells to death ligands

Invited Keynote lecture Abstract

Matthias Landgraf

Not just the bad guys – reactive oxygen species as novel regulators of structural plasticity in the nervous system.

Matthew C. W. Oswald[1], Paul S. Brooks[1], Maarten F. Zwart[2], Amrita Mukherjee[1], Ryan J. H. West[4], Carlo Giachello[3], Khomgrit Morarach[1], Richard A. Baines[3], Sean T. Sweeney[4], Matthias Landgraf[1]

[1] *University of Cambridge, Department of Zoology, Downing Street, Cambridge, CB2 3EJ, United Kingdom*
[2] *HHMI Janelia Research Campus, Ashburn, VA, 20147, USA*
[3] *University of Manchester, AV Hill (1.016), FBMH, Manchester, M13 9PT*
[4] *Department of Biology, University of York, Heslington York YO10 5DD, United Kingdom*

Reactive Oxygen Species (ROS) have been primarily regarded as damaging substances. For example, the influential 1950s "Mitochondrial Free Radical Theory of Aging" posited that ROS, generated as by-products of respiration and accumulating over time, lead to ageing and neurodegenerative conditions. We wanted to find out about the function of ROS in the nervous system under normal, non-pathological conditions. Our findings suggest a different view of ROS, which primarily function as signalling molecules important during normal nervous system development and function. Working with the *Drosophila* larva as a model, we identified ROS as novel regulators required for activity-regulated plasticity of nerve terminals, connectivity and behaviour. We identified the conserved Parkinson's protein DJ-1ß as a ROS sensor in neurons, and find that mitochondrial *vs* membrane generated ROS regulate different aspects of structural plasticity. This new framework facilitates targeted studies of how ROS might normally operate as metabolic feedback signals, and how their dysregulation with age and disease could initiate a vicious cycle of cellular stress leading to toxicity.

Petra Bleeker

"Natural variation in tomato specialised metabolites against insects"

Ruy Kortbeek[1], Marc Galland[1], Aleksandra Muras[1], Johan Westerhuis[1], Bart Andre[2], Lissy-Anne Denkers[1], Sasha van Hijum[3], Alain Tissier[4], Sebastien Zabel[4], Michel Haring[1], Robert Schuurink[1] & Petra Bleeker[1,2].

[1]: University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam The Netherlands
[2]: Enza Zaden Research and Development B.V. Enkhuizen, The Netherlands.
[3]: Radboud University Medical Center, Bacterial Genomics Group, Nijmegen, The Netherlands.
[4]: Leibniz Institute for Plant Biochemistry, Department of Cell and Metabolic Biology, Halle Germany.

With classical chemical insecticides progressively banned, understanding and deploying natural insect resistance found in (wild) ancestral relatives in order to protect our vegetable crops is gaining importance. Wild relatives of cultivated tomato (*Solanum lycopersicum*) display considerable resistance to agronomically important pest insects such as the Silverleaf whitefly and the Western Flower thrips, that vector a number of devastating plant-viruses. We aim to identify compounds produced by wild tomato that have anti-insecticidal properties.

In tomato, glandular trichomes are the "biochemical factories" responsible for production, storage and emission of specialised metabolites such as terpene (derivatives) and acylsugars, and some of these compounds have already been implicated useful in protecting plants against insects. We phenotyped a collection of 19 tomato accessions encompassing 10 different wild- and cultivated species for resistance against whiteflies and thrips and analysed their trichome's specialised metabolite profiles focusing on acylsugars and terpenoids. Logistic regression and survival modelling in combination with a "Random Forest Analysis approach, resulted in a number of compounds predicted to have (insect-specific) anti-insecticidal effects.

One of the compounds resulting from the prediction analysis is a derivative of glandular trichome produced 7-epizingiberene, a terpene we previously found to make tomato unattractive to whiteflies and spidermites. The effect of these terpenes was validated in a pure compound bioassay with whitefly and thrips. Moreover, we have elucidated the biochemical pathway leading to the production of these deterrent and toxic terpenoids and identified the (wild) tomato genes. However, introgression of these genes into a cultivated tomato background by backcrossing resulted in only very low levels of the compounds made, presumably by the inheritance of a dominant-negative factor from the cultivated side, a factor that remains as a subject of discussion and further study.

Oral Communication
Abstract

OC-1

From RNPs to memory: Imp and CamkII in activity-dependent remodeling

Nadia FORMICOLA(1), Marjorie HEIM(1), Ugur DAG(2), Krystyna KELEMAN(2) and Florence BESSE(1)

(1):Université Côte d'Azur, CNRS, Inserm, iBV, Parc Valrose, 06100, Nice, France; (2): HHMI Janelia Research Campus, Ashburn, VA, 20147, USA

Long-term memory (LTM) defines who we are, and is established via long-lasting changes at synapses. Specifically, de novo protein synthesis is needed for LTM to form, involving not only translation of newly-transcribed RNAs, but also experience-induced translation of quiescent mRNAs carried and stored at synapses. For their transport and translational control, mRNAs are packaged with regulatory RNA binding proteins (RBPs) into ribonucleoprotein particles (RNP). To date, the molecular trigger underlying activity-dependent neuronal RNP remodeling, and its impact on the establishment of LTM in vivo are unknown. Thus, characterizing RNP regulation will likely help better understand the origin and progression of developmental and age-related memory disorders, some of them being already linked to RBP dysfunction (e.g. Fragile X syndrome) or RNA repeat expansion.

To explore this process, we use as a paradigm RNPs containing the conserved RBP Imp in Drosophila. We showed that interfering with Imp function in a population of CNS neurons involved in learning and memory, dramatically impairs LTM. Hence, we aim at understanding the molecular bases of this phenotype, and studying the regulation of Imp RNPs in response to neuronal activity. We showed that depolarization and acetylcholine-mediated activation of neurons led to dramatic changes in Imp RNP size and morphology. A candidate regulator of such changes is CamkII, a conserved Ca2+-activated kinase which we identified as a partner of Imp in an IP-Mass-Spectrometry analysis. Our data show that the Imp-CamkII interaction is not mediated by RNA, but depends on CamkII activity and Imp prion-like domain, a domain known to be involved in RNP homeostasis. Our results also revealed that CamkII regulates both the morphology and dynamics of Imp RNPs in cultured cells. To study the impact of CamkII activation/inactivation on Imp RNP regulation, we are using a combination of genetic gain and loss-of-function in vivo approaches and cultured cell-based assays. Our data indicate that CamkII regulates the size, turnover and motility of Imp granules, suggesting its involvement in Ca2+-dependent, and possibly activity-dependent regulation of Imp RNPs leading to LTM formation.

OC-2

A targeted pharmacological approach for Voltage Gated Calcium Channels highlights the role of Cav2.1 in calcium flux deregulations in Fragile X neurons.

Sara Castagnola1,3, Agnes Paquet1, Sébastien Delhaye1,3, Alessandra Folci1, Fabrice Duprat2, Marielle Jarjat1,3, Mauro Grossi1,3, Méline Béal1,3, Stéphane Martin2, Frederic Brau1, Barbara Bardoni2,3 & Thomas Maurin1,3

1: Université Côte d'Azur, CNRS, IPMC, Valbonne - France. 2: Université Côte d'Azur, INSERM, CNRS, IPMC, Valbonne - France. 3: CNRS LIA « Neogenex », Valbonne - France.

Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and a leading cause of autism. FXS results from the loss of expression of the Fmr1 gene which encodes the Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein. FMRP directly binds numerous mRNAs encoding regulators of ion homeostasis, especially those implicated in calcium regulation, and it has also been shown to modulate ion channel activity through protein/protein interactions. FMRP is therefore likely to play critical roles in ion homeostasis and nervous system development. We carried out a functional analysis of Ca2+ homeostasis in FXS neurons using ratiometric calcium recordings in primary cultures. Our results show that primary neurons derived from mice lacking FMRP (Fmr1-KO) display impaired calcium homeostasis at several levels. Moreover, a targeted pharmacological approach of voltage gated calcium channels allowed us to show the critical role of Cav2.1 deregulation in FXS neurons. Consistently, we show that Cacna1a, the pore-forming subunit of Cav2.1 channels, is less expressed at the plasma membrane of Fmr1-KO neurons compared to WT. Finally, considering cell shape together with calcium constants, we could identify five cell types, identical in both WT and FXS primary neuron cultures. Therefore, our analysis describes the functional deregulation of Ca2+ homeostasis in FXS neurons and highlights a new phenotype of these cells that is amenable to drug screen.

OC-3

Hyperactivation of GABAergic neurons and cortical spreading depression: a new pathological mechanism of migraine?

Zerimech S., Chever O., Loucif A., Ayrault M., Duprat F., Cestèle S. and Mantegazza M.

1. Institut de Pharmacologie Moléculaire et Cellulaire, CNRS UMR7275, 2. Université Côte d'Azur

Hyperactivation of GABAergic neurons and cortical spreading depression: a new pathological mechanism of migraine?

Migraine is a highly prevalent episodic brain disorder with dramatic socio-economic implications. 30% of patients have transient neurological disturbances before the headache, called Aura.

Cortical spreading depression (CSD) is an extensively studied pathologic mechanism linked to the onset of the aura. However, specific mechanisms leading to CSD initiation are not completely understood yet.

Mutations of the Nav1.1 sodium channel (the SCN1A gene) cause Familial Hemiplegic Migraine type-3 (FHM3), a subtype of Migraine with Aura. They induce gain-of-function of Nav1.1 and hyperexcitability of GABAergic interneurons in culture. However no casual link has been demonstrated between the gain of function and the CSD, and it is still not clear how CSD could be generated by these dysfunctions.

We have identified a novel mechanism of CSD initiation, showing for the first time that optogenetic-induced hyperactivity of GABAergic neurons can ignite CSD in vivo in mice and ex vivo in brain slices.

Synaptic transmission is not necessary for CSD ignition, which is caused by neuronal spiking-induced extracellular K+ build-up. Consistently with the effect of FHM3 Nav1.1 mutations, hyperactivation of Nav1.1 with a specific activator (the toxin Hma1) can also ignite CSD.

We showed a causal relationship between initial hyperactivity of GABAergic neurons and CSD ignition. We have disclosed here a novel pathophysiological mechanism at play in FHM3 and possibly implicated also in other types of migraine.

OC-4

Post-translational-mediated Ryanodine Receptors calcium leak leads to Alzheimer's disease-like pathologies, and cognitive deficits.

Renaud Bussiere 1, Alain Lacampagne 2, Xiaoping Liu 3, Steven Reiken 3, Valérie Scheuerman 2, Ran Zalk 3, Andrew F. Teich 4 , Ottavio Arancio 4, Albano C. Meli 2, Inger Lauritzen 1, Nathalie Saint 2, Charlotte Bauer 1, Fabrice Duprat1, Cécile Martin1, Cla

1 Université Côte d'Azur, CNRS, IPMC, France, "Labex Distalz," 660 route des Lucioles, 06560 Sophia-Antipolis, Valbonne, France. 2 INSERM U1046, CNRS UMR9214, CNRS LIA1185, Université de Montpellier, CHRU Montpellier, 34295 Montpellier, France. 3 Departme

Background: Data are now converging to suggest an important contribution of Endoplasmic Reticulum (ER) Ca2+ homeostasis deregulation in Alzheimer's disease (AD) pathological process. This combines pathological ER Ca2+ release via the Inositol 1,4,5-trisphosphate and the Ryanodine (RyR) Receptors. Importantly, the molecular mechanisms underlying RyR2-mediated ER Ca2+ leak in AD were not fully understood. Materials and Methods: We studied post-translational remodeling of RyR2 in brains of AD patients, in two murine models of AD (3×Tg-AD and APP +/- /PS1 +/-), and in in vitro AD model (SH-SY5Y neuroblastoma cells expressing the harboring the familial APP with the double Swedish mutations (APPswe)). RyR2-mediated ER Ca2+ leak was investigated by imagery and single channel analyses. We used pharmacologic (stabilization of calstabin2 on RyR2 complex) and genetic approaches (RyR2 phosphorylated and unphosphorylated mutants) to modulate RyR2 macromolecular complex remodeling. Results: We showed that post-translational modifications (phosphorylation, oxidation, and nitrosylation) occur on RyR2 in human brains and in AD models [1, 2]. We identified the molecular cascade in which Amyloid ? (A?) activates ?2-Adrenergic receptors leading to neuronal RyR2 channels post-translational remodeling thereby enhancing the ER Ca2+ leak and activating Ca2+ dependent signaling pathways which contribute to AD pathogenesis [1, 2]. We also showed that pharmacological or genetic rescue of RyR2-mediated ER Ca2+ leak reduced A? load, normalized behavioral and cognitive functions and improved synaptic plasticity. [2] Discussion: Our data support the hypothesis that intracellular Ca2+ leak can be an early factor in the development of AD. Conclusions: Data from the present study and from others raise the possibility of a vicious circle in which leaky RyR2 channels promote A? production and A? enhances RyR2 leak. This study also provides a mechanism underlying leaky RyR2 channels, which could be considered as a potential therapeutic target for AD.

1. Bussiere R., et al. Amyloid β production is regulated by β2-adrenergic signaling-mediated post-translational modifications ofthe ryanodine receptor. J Biol Chem. 2017 Jun 16;292(24):10153-10168. doi:10.1074/jbc.M116.743070

2. Lacampagne A., Liu X., Reiken S., Bussiere R., et al. Post-translational remodeling of ryanodine receptor induces calcium leak leading to Alzheimer's disease-like pathologies and cognitive deficits. Acta Neuropathol. 2017 Jun 19. doi: 10.1007/s00401-017-1733-7.

OC-5

Study of UPR function in Parkinson's disease

EL MANAA W, DUPLAN E, GOIRAN T, CHAMI M, LAURITZEN I, CHECLER F, ALVES DA COSTA C

Institut de Pharmacologie Moléculaire et Cellulaire, Université Côte d'Azur, Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), Valbonne, France.

Parkinson's disease (PD) is a neurodegenerative pathology which shows motor symptoms including tremor, bradykinesia, rigidity and postural instability. It also involves non-motor symptoms such as cognitive impairment, mental manifestation, autonomic disorder and sensory disturbance. PD is characterized by the presence of intracellular inclusions, named Lewy bodies that are mainly composed of misfolded proteins such as alpha-synuclein. The accumulation of misfolded proteins is a molecular trigger of a cellular stress response in the endoplasmic reticulum (ER) called the Unfolded Protein Response (UPR). The modulation of the UPR has been demonstrated in post mortem studies of brain samples of patients affected by PD. Interestingly, even if, several PD-causative gene-products were shown to be linked to ER-stress, no study devoted to examination of the interplay between PD-causative gene product and UPR has been performed to date. The aim of our study was to address several questions. 1) Are PD-causative genes products modulated by ER stress? 2) Which is the mechanism responsible for PD associated proteins up-regulation in ER stress conditions? Indeed, I was able to show that PINK1 is up regulated by ER pharmacological ER stress both at mRNA and protein level ex-vivo and in vivo. The induction of PINK1 correlates with the activation of the three main arms of the UPR response and we are actually exploring the impact of each pathway to PINK1 regulation by means of genetic and pharmacological approaches.

OC-6

Direct reprogramming of cells into corticospinal motor neurons

Torsten Felske, Kawssar Harb, Christian Alfano, Michèle Studer

1 2 3 4 iBV

Loss of neurons is a major issue in mammals due to their inability to regenerate. The conversion of cells from any lineage into neurons, without passing by a de-differentiated state (direct reprogramming) holds great promises in cell-based repair of the central nervous system. That is due to fact that somatic cells from the patient itself can be reprogrammed which might help reducing the risk of transplantation rejection. Corticospinal Motor Neurons (CSpMN) in particular are of great interest as their absence or damage is the key cause for several neurological diseases e.g. Amyotrophic lateral sclerosis (ALS), cord lesions or stroke. To date, several types of neurons have been generated via direct reprogramming but only few studies managed to produce CSpMNs. Here I show that through ectopical expression of the transcription factor Fezf2, together with the nuclear co-adaptor Lmo4, neural cells can be directed to acquire a molecular identity characteristically of CSpMNs in vivo and in vitro. Transfection of Fezf2 and Lmo4 in early-born neurons of a different subtype in the neocortex of embryonic mice directs these cells to express Ctip2, a prominent CSpMN marker and drives the projection of their axons towards the spinal cord. Furthermore, this conversion is observed also in developed mature neurons of new-borns, suggesting a powerful reprogramming potency of these factors. Additionally, in vitro studies demonstrate that early mature astrocytes can also be reprogrammed, as assessed by neuronal morphology and expression of Ctip2. Overall, our investigations unravel a powerful combination of Fezf2 and Lmo4, to efficiently reprogram cells from other lineages into CSpMNs.

OC-7

Alternative translation initiation as a molecular mechanism of K2P potassium channel dysfunction in Migraine

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Migraine is a disabling neurological disorder with an annual prevalence estimated at ~15%. In humans, 2 mutations have been found to produce a dominant negative for TRESK, a two-pore-domain K+ channel implicated in migraine: TRESK-MT, a 2 bp frameshift mutation (F139WfsX24) and TRESK-C110R, a missense mutation. Despite the fact that both mutants strongly inhibit TRESK, only TRESK-MT leads to an increase in sensory neuron excitability and is associated with a migraine phenotype. Here we find that the frameshift TRESK-MT mutation induces alternative translation initiation (ATI) which leads, from the same mRNA, to two proteins: MT1 and MT2. We show that by co-assembling with and inhibiting TREK, another subfamily of K2P channels, MT2 increases trigeminal sensory neuron excitability, a key component of migraine induction. This finding identifies TREK as major molecular target in migraine pathophysiology and treatment and resolves the contradictory lack of effect of TRESK-C110R which targets only TRESK and not TREK. In addition, we report a new migraine-associated TRESK mutant (Y121LfsX44) that also produces two fragments with differential effects on TRESK and TREK, providing further evidences for the involvement of frameshift mutation inducing ATI (fsATI) in a major human disorder. Therefore, fsATI mechanism needs to be considered when analyzing frameshift mutations and motivates future work to re-analyze frameshift mutations linked to human disorders.

OC-8

Sexual dimorphism of the adrenal cortex is driven by hormone dependent activation of distinct stem cell compartments

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The impact of gender on tissue homeostasis has thus far been poorly described, despite the fact that disease prevalence has been shown to vary between sexes. The adrenal gland is no exception and women are up to 6 times more frequently diagnosed with adrenocortical cancer than men. My study demonstrates sexually dimorphic tissue renewal in the adrenal cortex, as shown by lineage tracing experiments and proliferation analysis. Lineage tracing using the Axin2-creERT2 mouse line highlighted increased turnover in the female cortex, which was completely replaced in less than 4 months. Furthermore, using the Gli1-CreERT2 mouse line, we identified female specific recruitment of capsular stem cells to the adrenal cortex in adults. Proliferation quantification confirmed that the capsule, zona glomerulosa and zona fasciculata tissues had higher proliferation rates in females than males. To address the origin of this sexual dimorphism, we made use of sex reversal mouse models and showed that the hormonal sex, rather than the chromosomic sex is driving the adrenal cortex sexual dimorphism. To our knowledge, this is the first model of sex-specific stem cell regulation in a non-reproductive organ.

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OC-9

Rspo1 in Female Sex Determination

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Key words: Rspo1, Wnt/beta-catenin signaling, ovarian development, gonadal somatic cells, sex reversal

Rspo1-4 genes encode secreted proteins that can activate the canonical Wnt/beta-catenin signaling pathway. Mutations in Rspo1 or Wnt4 lead to female to male sex reversal of XX individuals, both in humans and mice. Rspo1 mediated activation of the canonical Wnt/beta-catenin signaling is required both for ovarian development and to repress the testicular male differentiation pathway. However, the precise timing of this requirement during gonad development is not well defined.

We have produced a new floxed Rspo1 allele (Rspo1F) for conditional deletion of Rspo1 in the XX gonadal cells at different stages. To validate this new mouse model, we have first generated a constitutive knockout, Rspo1 deleted (RSPO1D), by crossing Rspo1F mice with Sox2-CreTg/+ mice (Rspo1 deletion in the zygote). As expected from an RSPO1 loss of function, XX Rspo1D/D gonads show female to male sex reversal phenotypes. Namely, total deletion of Rspo1 releases the female pre-granulosa cells from mitotic arrest and leads to their precocious differentiation prior to trans-differentiation towards the male Sertoli cell fate. In addition, only a few germ cells survived in the posterior gonad of XX Rspo1D/D animals.

To understand the precise timing of Rspo1 requirement during gonad development, we have used the Sf1-Cre transgene to delete Rspo1 specifically in the gonadal somatic cells around E11.5. We have not observed female to male sex reversal, indicating that RSPO1 might functionally affect female sex determination at early stages of gonad development before the activation of the Sf1-Cre transgene, or in other gonadal cells not targeted by this transgene such as the germ cells and some epithelial cells.

We have also established an inducible Rspo1 conditional mouse model by using the knock-in gene Wt1CreERT2, in which CRE activity and the subsequent deletion of Rspo1 is induced by tamoxifen. We have found that tamoxifen induction at E9.5 and E10.5 results in female to male sex reversal, unlike tamoxifen induction at E11.5 and E12.5. These results suggest an early requirement for RSPO1 function in the XX gonad. We will next perform RNA-seq on Rspo1 mutant gonads at the several stages to find out the downstream target of Rspo1 and further address the mechanism behind female to male sex reversal.

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OC-10

Analysis of Sox8 and Sox9 in XX R-spondin1 Sex Reversal

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In mammals, testis development requires SRY-dependent activation of SOX9 and ovarian development involves R-spondin1 (RSPO1) mediated WNT/beta-catenin signaling. Indeed, male (XY) mouse gonads lacking Sox9 develop as ovaries, and female (XX) gonads lacking Rspo1 develop as a partially sex-reversed ovo-testis. Interestingly, XY and XX gonads lacking both Rspo1 and Sox9 also develop as an ovo-testis. Testicular development in XX Rspo1 Sox9 double mutant mice is especially intriguing since the gonads genetically lack both Sry and Sox9. This suggests that a factor can replace Sox9-dependent testicular development. Since genetic studies show that Sox8 supports the function of Sox9, we hypothesize that testicular development in Rspo1 Sox9 double mutant mice depends on SOX8. To test this, we aimed to study gonad development in Rspo1 Sox8 Sox9 triple mutant mice. As part of this study, we also investigated gonad development in Rspo1 Sox8 double mutant mice. We found that XX Rspo1 Sox8 gonads developed as an ovo-testis which indicates that like Sox9, Sox8 is dispensable for XX Rspo1 sex reversal. In XX Rspo1 Sox8 Sox9 triple mutant gonads, we detected rare cells which express DMRT1, a testis Sertoli cell marker. This sharply contrasted DMRT1 expression in XX Rspo1 Sox9 sex reversed gonads. Though quantification by RT-qPCR is required, the preliminary data shows that ablation of Sox8 dramatically impairs testis development in XX Rspo1 Sox9 mice. Thus Sox8 and Sox9 appear to be jointly required for sex reversal in Rspo1 mice. The findings of this study may be of relevance to SRY-negative XX individuals where SOX9 does not appear to have a role in female-to-male gonadal dysgenesis.

OC-11

Determine the role of the WNT/β-catenin signaling pathway in germ cell differentiation.

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Taking advantage of our expertise in the analysis of gonadal differentiation models, we aim to determine the role of the WNT/?-catenin signaling pathway in normal and pathological differentiation of the gonad. To do this, we will 1) use ?-catenin loss-of-function murine models to analyze the consequences of modifying its expression in each cell type; 2) determine how ?-catenin regulates the expression of pluripotency genes in germ cells; and 3) examine how WNT/?-catenin signaling in germ cells influences the differentiation of their somatic environment. My lab identified a mutant R-spondin1 (Rspo1) gene to be responsible for XX to XY sex reversal in humans. These individuals are genetically female, but develop as male. To understand Rspo1, we developed a Rspo1 knock-out mouse model. The protein encoded by Rspo1 controls the activation of canonical WNT/?-catenin signaling, an essential pathway in ovarian development. My thesis project is to determine the role of WNT/?-catenin signaling in each cell type in the developing mouse ovary in vivo. Using mouse models, we now show that, in XX germ cells, Ctnnb1 gene (encoding ?-catenin) has been conditionally deleted, proliferation and differentiation are all impaired. Moreover, using XX gonads in which Ctnnb1 has been deleted in somatic cells, we also show that the somatic cells are required for germ cells to differentiate and enter into meiosis. We demonstrate that ?-catenin signalling controls primordial germ cell pluripotency in the mouse ovary.

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OC-12

Analysing the role of the E3-Ubiquitin ligase Mindbomb1 in Planar Cell Polarity signalling

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Planar Cell Polarity (PCP) is the collective alignment of cell polarity across the plane of the tissue. Upregulation of PCP components is observed in many cancers and this is associated with a poor prognosis of patients. One of the most highlighted roles of PCP deregulation is in cancer cell invasion and metastasis. Core PCP components are asymmetrically localised in a migrating cancer cell. These asymmetrically distributed components can regulate cytoskeletal remodelling and cell contractility. Thereby, PCP can facilitate efficient cell invasion and metastasis. However, mechanisms that regulate asymmetric distribution of these core components are poorly understood.

We are using Zebrafish gastrulation as an in vivo model system to study the regulation of PCP. I found that embryos depleted of Mindbomb1 (Mib1), an E3-ubiquitin ligase necessary for Delta-Notch signalling, have neural tube defects similar to a PCP component mutant called Vangl2. These findings suggest that beyond its well-known role in Delta/Notch signalling, Mib1 may represent as a novel crucial regulator of the PCP pathway.

PCP pathway regulates convergent extension (CE) of the embryonic axis during gastrulation. I showed that Mib1 morphant embryos exhibit a reduced CE, suggesting that Mib1 acts a general regulator of PCP-dependent morphogenetic processes. Interestingly, this function of Mib1 in CE is entirely independent of Delta/Notch signalling. CE defects of Mib1-depleted embryos can be rescued by a PCP effector protein RhoA, indicating that Mib1 is affecting CE movements through a regulation of Wnt/PCP pathway activity. Further functional assays showed that the E3-ubiquitin ligase activity of Mib1 is necessary for CE movements.

These findings suggest that Mib1 may regulate embryonic morphogenesis by promoting the ubiquitination of a PCP component. The Wnt co-receptor Receptor-like Tyrosine Kinase (Ryk), which interacts with Mib1 in C.elegans and controls Wnt5/PCP signaling in Zebrafish was the obvious target for us study it's interaction with Mib1. We have shown that Mib1 indeed regulates the internalization of Ryk from the membrane to regulate the PCP pathway.

OC-13

Role of LRRC8/VRAC in Epithelial-to-Mesenchymal Transition (EMT)

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Reactive Oxygen Species (ROS) are a family of very active molecules that have a major signaling role, involved in a large variety of processes such as embryogenesis, Epithelial-to-Mesenchymal Transition (EMT), organ fibrosis and cell death. In order to control ROS signaling events, cells need to balance their production and reactivity with antioxidants such as glutathione (GSH, the major intracellular antioxidant). LRRC8s are a family of proteins that form the Volume-Regulated Anion Channel (VRAC), an ion channel mainly permeable to chloride but also to larger anions. In HEK293 cells, we have demonstrated that LRRC8/VRAC is permeable to GSH and that VRAC activation drives a leak of GSH. This GSH decrease is blocked by the VRAC inhibitor DCPIB and is not present in cells lacking LRRC8/VRAC (CRISPR/Cas9). To validate the relevance of the GSH permeability of LRRC8/VRAC, we have developed an EMT model in renal proximal tubule epithelial cells under the dependence of ROS. Indeed, in HK-2 cells, the pleiotropic growth factor TGF-?1 stimulates ROS production and GSH decrease, which secondarily drives the EMT, as evidenced by changes of cell morphology and EMT markers expression, assessed at both gene and protein level. As expected, inhibition of LRRC8/VRAC by DCPIB prevented TGF-?1-induced GSH depletion, ROS production, and attenuated EMT response. These results suggest that LRRC8/VRAC, by its ability to modulate ROS levels, plays a critical role in EMT.

OC-14

CCL5 Protects Against The Development Of Obesity, Diabetes And Associated Neuropathic Pain.

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CCL5/RANTES, a chemoattractant cytokine, is well known for its role in cerebral and peripheral inflammation. Together with its receptors CCR1, CCR3 and CCR5 it also contributes to neural function and diseases such as obesity, type 2 diabetes (T2D) and neuropathic pain.

Herein, we investigated the role of CCL5 in the development of diet-induced obesity (DIO), metabolic impairment and neuropathic pain.

We tested the long-term effects of high-fat (HFD) or standard diet on the development of obesity in adult CCL5-/- mice and wild-type mice (WT) and discovered that CCL5-/- mice seem to be protected from weight gain and the associated impairment of glucose metabolism.

Furthermore, CCL5-/- mice show a different expression pattern of neuropeptides and inflammatory markers compared to control mice. To evaluate the implication of CCL5 in neuropathic pain associated with diabetes, thermal pain sensitivity of CCL5 -/- mice was measured in both conditions. Remarkably, CCL5 -/- displayed higher tolerance to pain in HF condition compared to control mice.

Our results indicate that the absence of CCL5 seems to have a protective effect on the development of obesity and associated metabolic impairment as well as an ameliorating effect on thermal pain sensitivity under a HF challenge. Thus, CCL5 could be involved in the maintenance of overfeeding, through indirect action on neuronal hypothalamic systems, in the deregulated central control of energy balance found in obesity.

OC-15

Effect of T cell specific PPAR? overexpression on diet-induced obesity and its associated inflammation and insulin resistance

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Background and aims: It is now recognized that obesity-induced chronic inflammation is central to the development of many obesity-associated pathologies such as type-2 diabetes. Obesity-induced T cell changes in adipose tissue (AT) has been linked to metabolic phenotypes of obese subjects. Interestingly, it has also been shown that T cell differentiation and responses may be altered by modulating their metabolism. Accordingly, we have recently shown that Peroxisome-Proliferator-Activated-Receptor beta (PPAR?) increases lipid metabolism in T cells, which changes T cell homeostasis. Our objectives were to study the consequences of changes in T cell populations induced by T cell-specific PPAR? overexpression on obesogen high fat diet (HFD)-induced AT inflammation and the subsequent development of insulin resistance (IR).

Materials and methods: We created transgenic mice that overexpress PPAR? specifically in T cells (Tg T-PPAR? mice) using the Cre-Lox system. 12-week old male mice were fed with HFD (60% of calories derived from fat) during 16 weeks. Insulin- and glucose-tolerance tests (ITT/GTT) were performed at week 11 and 13 of the diet, respectively. At the end of the 16-week diet, AT (subcutaneous and epididymal) stromal vascular cells were isolated for analysis of the presence of macrophage and T cell populations using flow cytometry. Liver steatosis was analyzed by histology and triglyceride measurements.

Results: HFD-induced weight gain was 8% less in Tg T-PPAR? compared to control mice. ITT/GTT data showed that Tg T-PPAR? mice develop less IR than control mice, even in weight-matched mice. Total AT macrophage numbers were decreased predominantly due to a decrease in pro-inflammatory Ml macrophages. Furthermore, AT T cells numbers were also decreased due to a decrease in a? T cells, while ?? T cell numbers remained unchanged. Together, this led to an increase in the proportion of ?? T cells in AT. Liver weight and triglyceride content was decreased.

Conclusion: T cell-specific overexpression of PPAR? results in: 1) a partial protection against HFD-induced obesity, insulin resistance, and liver steatosis, 2) a reduction in AT inflammation, 3) an increase in the proportion of ?? T cells in AT. Our hypothesis is that the beneficial effects observed are the consequence of a decrease in ?? T cells (leading to an increase in ?? T cell prevalence) as the result of changes in T cell metabolism induced by PPAR? overexpression.

OC-16

Brown-like adipocyte progenitors derived from human iPS cells: A new tool for anti-obesity drug discovery and cell based therapy

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Obesity and associated metabolic disorders such as diabetes and cardiovascular diseases are major health problems. Obesity results from an imbalance between calorie intake and energy expenditure, and three types of adipocytes are the main regulators of this balance. White adipocytes are involved in energy storage and their accumulation marks obesity. In contrast, classical brown adipocytes and brown-like adipocytes (BAs) dispersed in white adipose tissues are specialized in energy expenditure. Upon activation, BAs consume metabolic substrates and burn fat via the mitochondrial uncoupling protein-1 (UCP-1). Therefore, BAs represent promising cell targets to counteract obesity. However, the scarcity of BAs in adults is a major limitation for a BA-based therapy of obesity, and the notion to increase the BA mass by transplanting BA progenitors (BAPs) in obese patients recently emerged. Following the pioneer work of Yamanakaâ€™s group on the generation of patient-specific induced pluripotent stem cells (iPSCs) by reprogramming somatic cells, hiPSCs emerged as an abundant source of cells of therapeutic interest. We were among the first to report the potential of hiPSCs to generate BAPs, which are able to differentiate at a high efficiency.

Furthermore, tissue engineering aiming to develop tissue-like structures that mimic the in vivo situation. Cells are classically grown as monolayer, which poorly reflects the in vivo situation. In the contrast, the cell-cell and cell-extracellular matrix interaction are promoted in 3D configurations. Therefore, 3D cultures represent a bridge between traditional cell culture and live tissue to overcome the limitation that impaired discovery of efficient and safe drugs.

We have established conditions to generate hiPSC-BAPs 3D aggregates, also known as spheroids, able to differentiate into UCP-1 expressing adipocytes, therefore named as adipospheres. The differentiation efficiency compared to the classical 2D culture will be shown. The adiposphere model has been improved with the enrichment of endothelial cells to better mimic the adipose tissue microenvironment.

This cell model represents an unlimited source of human BAPs that in a near future may be a relevant tool for both therapeutic transplantation and for the discovery of novel efficient and safe anti-obesity drugs. It should also allow a better understanding of the interactions between different cell types of adipose tissue for BAs recruitment and activation.

OC-17

Deciphering the adipose tissue browning in physiopathological contexts

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Adipose tissue regulates energy homeostasis and is one of the most adaptive tissues in the organism. White adipose tissue (WAT) stores energy as lipids. Brown adipocytes from the brown adipose tissue (BAT) and brown-like (beige) adipocytes are cells known to expand energy into heat thanks to the uncoupling protein 1 (UCP1). UCP1 induction is controlled by several pathways including p38 signaling, turning then WAT into beige tissue. In adults, brown/beige adipocytes are sparse and they disappear with age. In contrast, browning process is reactivated in pathologies such as the cancer-associated cachexia. Knowing the key molecules involved in the induction of the brown markers especially UCP1 is of interest to identify the mechanisms controlling brown adipocytes formation and the role of energy expenditure in physiopathological conditions.

Aging is a physiological process resulting in the loss of brown adipocytes; Therefore, we hypothesize that using an anti-aging molecule (resveratrol) could induce UCP1 expression and the browning of the adipose tissue. We noted that high doses of resveratrol are increasing Sirtuin 1 (SIRT1) mRNA, an anti-aging enzyme but not UCP1 expressions. However, low doses of resveratrol stimulated UCP1 expression but not SIRT1. Induction of UCP1 by resveratrol was blunted by SB203580, a p38 inhibitor. Thus, a decrease in UCP1 expression related to the aging process cannot be directly correlated with SIRT1 expression.

In contrast, when breast cancer stem cells were grown as mammospheres and co-cultured with hMADS-adipocytes, we observed a decrease in the size of the lipid droplets for the adipocytes in direct contact with the mammospheres. Furthermore, by using a GFP reporter model for UCP1 expression, we noticed that these adipocytes in close contact with the mammosphere expressed GFP. Further experiments are necessary to assess the relevance of this observation and to identify how mammospheres induce browning of the cancer-associated adipocytes.

Overall, our results indicate that the browning process is induced in several processes and they bring new light on the associated molecular pathways involved.

OC-18

Microrna involvement in the control of brown adipose tissue activity in a model of predisposition to type 2 diabetes

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In humans and animals, maternal malnutrition predisposes offspring to diabetes with aging. In the maternal low protein (LP) diet model, the rat progeny suffers from age-dependent deterioration of glucose homeostasis. We showed that brown adipose tissue (BAT) is a decisive determinant of metabolic dysregulation in LP progeny. We hypothesized that microRNAs (miRNAs) could control BAT adaptation in LP progeny during aging.

Therefore, we performed a miRNA profile in BAT of young and old LP progenies. In young LP progeny with hyperactive BAT and normoglycemia, 58 over the 723 miRNAs screened were misexpressed and most of them were upregulated. Importantly, when BAT activity decreased and hyperglycemia appeared with aging, the expression of misexpressed miRNAs dropped to control levels. Since young LP rats displayed increased urinary noradrenaline, we performed a miRNA profile in BAT of rats exposed to chronic adrenergic treatment to activate BAT. Both adrenergic and LP rats showed hyperactive BAT with upregulated miRNAs, but only 3 miRNAs were commonly dysregulated. This suggests that the adrenergic pathway is unlikely to be involved in the miRNA upregulation in the LP BAT.

Bioinformatic analyses focused on metabolism decreased the number of miRNA candidates to 10. The in vitro validation of these miRNAs in primary culture of mature adipocytes allowed me to identify a promising candidate. I intend to complete my work by deciphering the signaling pathway targeted by this miRNA in a context of BAT activation. I will also investigate its role in the regulation of adipocyte differentiation and I will validate the biological role of this candidate in vivo. To do this, the candidate miRNA will be directly injected into the BAT of control animals or LP animals. Taken together, this work will reveal potential new therapeutic targets for prevention and/or treatment of type 2 diabetes.

OC-19

Possible role of miRNAs in the adaptation of hepatocytes to amino acid deprivation

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MicroRNAs (miRNAs) are small molecules containing 20-22 nucleotides, the biogenesis of which is governed by a complex protein network including Dicer, Drosha and Ago2. MiRNAs function as major regulators of gene expression in many biological programs by binding the 3â€™UTR region of mRNA targets, thus interfering with mRNA translation or its degradation. More recently it has been suggested that miRNAs buffer fluctuations in gene expression generated by perturbations inside organisms or coming from their environment.

We previously showed that amino acid restriction (aaR) during organismal development results in the upregulation of most of the miRNAs in fetal pancreas and fetal liver which occurs together with a decrease in proliferation of fetal ?-cells and fetal hepatocytes, respectively.

Our working hypothesis is that, in the context of a lack of aa availability, miRNAs could modify gene expression to help cells to deal with such environmental perturbation.

To delve into the mechanism of miRNA upregulation linked to nutrient restriction, we cultured primary rat hepatocytes with different aa concentrations. When primary rat hepatocytes are exposed to total aa deprivation (aaD), we observed a strong increase in Drosha protein level accompanied by an augmented level of several miRNAs. We also found that aaD blocks primary rat hepatocyte proliferation. To be specific, cells enter in a quiescence state characterized by a switch in cell metabolism, from oxidative phosphorylation to anaerobic glycolysis, and this correlates with an extension in hepatocyte lifespan. The restoration of aa in the culture medium is able to rescue cell proliferation and restore Drosha protein level, suggesting that aaD induces reversible cell adaptation mechanisms. Remarkably, Drosha knockdown by siRNA can prevent the block of cell proliferation induced by the absence of aa.

Given recent studies showing that Drosha is implicated in the transcriptional regulation of many genes independently from its role on miRNA biogenesis, we are currently investigating its relationship with the miRNA upregulation we observe.

We anticipate that our research will not only provide novel information in the field of basic miRNA biology, but it will likely also generate translational insights concerning nutritional regulation of health and its demise in disease situations.

OC-20

Amyloid-beta independent implication of the C99 fragment in Alzheimer's disease: study of a new Alzheimer's mouse model based on viral expression.

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Alzheimer's disease (AD) is the most common neurodegenerative disease and cause of dementia in the elderly. It induces the progressive loss of cognitive functions, and so far no definitive cure exists. AD is characterized by the accumulation and aggregation of Amyloid ? (A?) in the brain, which is thought to be one of the main contributors to the pathology. In our laboratory, recent study of a mouse model of AD (3xTg) shows that the precursor of A?, the C99 fragment, accumulates in neurons long before A? is detected (Lauritzen et al., 2012). This C99 accumulation correlates better with the progressive decline in synaptic and cognitive function than A?. Therefore, to focus on C99, we used an adeno-associated viral strategy to directly express C99 under the control of the synapsin promoter in wild-type mouse brains. 6 months after injection, C99 is expressed mainly within the hippocampus and cortex, in which it is localized to neuronal membranes. However, a subset of neurons also displays lysosomal-associated C99 staining corresponding to aggregated forms of C99. These mice also produce A? and develop synaptic plasticity alterations (LTP decreases) and cognitive deficits (memory loss). A? is produced from C99 by ?-secretase cleavage, thus to discriminate between C99 and A?, we treated some mice with a ?-secretase inhibitor, ELND006 (D6). We observed a decrease in A?, but also a strong increase in C99 localized to both pre-synaptic regions and lysosomes. In future studies, D6 will be used to evaluate whether C99 itself, in absence of A?, is able to induce synaptic and cognitive dysfunctions.

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OC-21

Epstein-Barr Virus infection in gingival tissues : towards new paradigm in oral pathogenesis

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The emergence of pathogenic bacterial or fungal species is mainly responsible for severe oral inflammatory condition occurring within the gingiva is a consensus in oral pathogenesis. However, a recent model proposes a synergistic mechanism combining viruses and bacteria to increase oral inflammatory condition and disease progression1.

EBV is a ubiquitous virus with oral persistence, its presence in the saliva and oral neo-infections events are major elements in its biology. However, its role in oral pathology is yet poorly known2,3.

In this context, we performed investigations to assess the presence of EBV in chronic periodontitis (CP) a inflammatory disease responsible of tooth loss and associated with several systemic diseases and in oral lichen planus (OLP) an autoimmune-like disease of unknown pathogenesis with a malignant potential.

PCR-based analysis and staining of EBV-infected cells in periodontal material from CP-patients demonstrated that EBV-infection worsens inflammatory condition and CP severity4.

We also established a link between the decrease of EBV load in saliva and the improvement of clinical feature after conventional CP treatment5.

Moreover, using in-situ hybridization, EBV-infected cells were sought in OLPs. We observed that OLPs are commonly infected with EBV and that a higher level of EBV-infection of OLP correlates with the severe clinical form of OLP (erosive OLP) and with a deeper inflammatory infiltrate. Interestingly, most infiltrated EBV-infected cells were mature B lymphocytes (CD20-;CD138+) also named plasma cells (PC). Finally, we showed that PCs produce EBV viral particles by electron microscopy6.

Increased presence of EBV in gingival inflammatory condition pave the way to possible EBV contribution in CP and OLP. Whether presence of EBV-infected PCs with an immunosuppressive activity through IL-10 secretion in OLP and CP may suppress some immune function and favor the emergence of pathogenic bacteria to fulfil with a synergistic model is an interesting question for the future7.

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OC-22

Micro-organ discovered to be a potential electroceutical for inflammatory disorders

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SignaLife

There is a micro-organ in the body that regulates blood pressure, glycemia and inflammation. Recent evidence has shown that hypertension and type 2 diabetes can be prevented by the activation of this micro-organ. This activation is via electrostimulation, which is electrical activation of a peripheral nerve using surgically implanted devices - otherwise known as electroceuticals. The aims of this project are: to determine if the principal nerve that connects to this micro-organ can attenuate inflammation via electrostimulation; to discover how electrostimulation can decrease inflammation and reveal if the targeted nerve can become an electroceutical for inflammatory disorders â€“ similar to hypertension and type 2 diabetes. A specialised surgery for isolating this micro-organ and its nerve was created, as well as a micro-electrode for the stimulation of this nerve. C57BL/6 mice, 8-10 weeks old, were used for this surgery, stimulated at a specific frequency and their blood was collected for analysis by multiple assays. In one specific instance, the adrenals were also bilaterally excised. Electrical stimulation of this nerve, after injection of LPS, attenuated the expression of a wide variety of inflammatory markers â€“ TNF, IL-1?, IL-6 and others. Additionally, the stimulation increased the expression of corticosterone â€“ an anti-inflammatory hormone. Bilateral excision of the adrenal glands prevented the effect of electrostimulation and the concentration of pro-inflammatory cytokines were unchanged. Mifepristone, a blocking drug for corticosteroneâ€™s receptor (glucocorticoid receptor), also blocked the effect of electrostimulation. Finally, a genetic strain of mice (LysMcre GRloxp) who lack glucocorticoid receptor on macrophages were obtained. When stimulated these LysMcre GRloxp mice showed no benefit of the electrostimulation and inflammatory markers were not decreased. Ultimately, Electrostimulation attenuates inflammation via the effect of corticosterone on macrophages. This proves the micro-organ and its nerve may be a potential electroceutical and could treat inflammatory disorders such as inflammatory bowel disease and arthritis.

OC-23

Effect of natural uranium on osteocyte mineralisation function and autophagy

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Consecutively to a variety of anthropic activities which led to an increasing environmental uranium background concentration (mainly due to massive phosphated fertilizer use), the general population is exposed to a daily intake of low doses of natural uranium through food and water ingestion. Kidney remains the first target of this heavy metal but bone is the final organ where it accumulates. Natural uranium has a low specific activity of 26 Bq/mg, thus it's toxicity must be considered more as a low dose chemical contaminant than a radiological compound. Uranium heterogeneously incorporates in bone, and preferentially in remodelling zones. Our group has recently demonstrated that uranium can impair in vitro the function of osteoblasts (Pierrefite-Carle V, et al,Arch Toxicol. 2017, Apr;91(4):1903-1914), as well as osteoclasts (Gritsaenko T, et al, Biochim Biophys Acta. 2017 Apr;1861(4):715-726). We decided to investigate the effect of natural Uranium on Osteocytes, the longest bone living cells acting as mechano-sensors. Despite the low doses ingested per day,accumulation and long lasting retention of uranium in bone is indeed more susceptible to impact these cells responsible for the orchestration of the bone remodeling process. This presentation will adress the results of the acute and chronic effects of natural Uranium on osteocyte differenciation and mineralisation function using cellular and molecular approaches on the Murine-Like-Osteocyte A5 (MLO-A5) cell line. Data showing the effect of this heavy metal on autophagy function will also be presented.

OC-24

Role of Perlecan in BMP signaling pathway

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The Basement Membrane (BM) is a specialized extracellular matrix that surrounds organs and acts both as a solid support and as a signaling platform for these organs. During development, the BM has to adapt to the volume and shape modifications of tissues to which it is associated. This implies a modulation of the amount of deposited BM and probably of its composition. Since growth factors regulate organogenesis, they could directly control this modulation, but whether a link between these signaling molecules and the regulation of the synthesis of BM components exists remains totally elusive.

Perlecan (Pcan), the main heparan sulfate (HS) proteoglycan of the BM, has multiple putative isoforms in all species. It can interact with growth factors via its HS chains. But little is known about the in vivo functions of Pcan and its isoforms in growth factors signaling. Consequently, using drosophila as a model system, I have initiated a work on Pcan isoformsâ€™ function in growth factor signaling pathways. I have characterized pcan transcriptional isoforms in the two tissues that are the source for Pcan in the wing imaginal disc (the disc itself and the adipose tissue). I have then shown that BMP in the wing imaginal disc regulates the transcription of pcan isoforms in this tissue. I have also demonstrated that Pcan is a repressor of the BMP signaling pathway in the wing disc. However, I still need to identify the isoform responsible for this repression. In conclusion, my data could provide crucial information for the understanding of the aetiology of BM related diseases.

OC-25

Active Sonic Hedgehog pathway is required for the airway multiciliated cell differentiation

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The airway epithelium is composed by multiciliated cells (MCCs), basal cells, and goblet cells. MCCs project at their apical side hundreds of motile cilia which beat in a coordinated manner to orchestrate the mucociliary clearance. In Cystic Fibrosis, the airway epithelium undergoes remodeling characterized by a goblet cell hyperplasia and a progressive loss of MCCs, leading to airway cleansing defects. A better characterization of molecular mechanisms involved in MCC differentiation (multiciliogenesis) represents a major issue with biomedical relevance. In previous works, we revealed that multiciliogenesis is controlled by complex interactions between the Notch and BMP pathways with miR-34/449 microRNAs. The Sonic Hedgehog (SHH) signal is another key pathway closely related to Notch and BMP pathways during development. Whereas the SHH signal is well described to contribute to the formation of the primary cilium, its role in multiciliogenesis remains unexplored. Here, we examined the role of the SHH pathway in MCC differentiation.

We used a 3D model of primary cultures of human airway epithelial cells (HAECs) to analyze RNA expression (RNA-seq and qPCR), and protein detection (western blot and immunostaining). Then, we examined the effects of pharmacological inhibition of the SHH pathway on MCC differentiation.

First, we showed that several key SHH members (SHH, SMO, PTCH1, SUFU and GLI3) as well as GLIS3, a poorly characterized transcription factor related to BMP and SHH pathways, were expressed during HAEC differentiation. Immunostaining experiments revealed that the ligand SHH, the receptor smoothened (SMO) and GLIS3 were enriched in the apical surface of MCCs, at the vicinity of motile cilia. Finally, we showed that a pharmacological inhibition of the SHH pathway using SMO antagonists dramatically affected the MCC formation during the airway epithelium regeneration.

In conclusion, our data indicate that active SHH pathway is required for MCC differentiation.

OC-26

The Ma gene for resistance to root-knot nematodes: insights into its unique features among TIR-NBS-LRRs

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Plant parasitic nematodes are major pests for agriculture. They cause huge losses worldwide estimated at hundreds of billions dollars per year. Among them, root-knot nematodes (RKNs) are the number one threat owing to their ability to infect over 5500 plant species. With the removal of pesticides and the constraints of the biological control, natural genetic resistance is a promising alternative strategy. This approach mainly relies on plant immunity factors, partly represented by the toll-interleukin-1-receptor (TIR), nucleotide-binding-site (NBS) and leucine-rich-repeat (LRR)-containing receptor proteins (TNLs), which specifically recognise secreted pathogen effectors and induce effector-triggered immunity. To date, the Ma TNL gene in plum is the sole gene conferring a sustainable and complete resistance to all RKNs in plants. Interestingly, this gene displays a peculiar structure among TNLs. We characterised its structure and evaluated its implication in the resistance process by annotating firstly the TNL family in peach, the reference genome for Rosaceae. We showed that the 194 peach TNL genes were mostly organised in clusters and highlighted the presence of an unknown C-terminal domain, designated the post-LRR (PL) domain. We showed that this domain is exclusive to TNL genes and found it in a large majority of TNLs from peach along with other plant genomes examined. Our data indicate that Ma is the only gene that displays five PL domains. The second aim of our study was to characterize the RMja TNL gene from almond that confers resistance to fewer RKN species. Using high-resolution mapping and genome sequencing, we showed that RMja is actually the Ma orthologue and displays a similar atypical architecture with extra PL domains. Finally, we shed light on polymorphic regions that might be involved in the RKN resistance spectrum. This study paves the way to reveal an original immune process for RKN control.

Van Ghelder C, Esmenjaud D. 2016. TNL genes in peach: insights into the post-LRR domain. BMC Genomics 17: 317

OC-27

WEE1 checkpoint control is necessary for a proper Root-Knot Nematode feeding site development

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Plant-parasitic nematodes are among the most devastating plant pathogens. Root-knot nematodes (RKN; Meloidogyne spp.) infect plant roots and trigger the formation of specialized feeding sites named â€œgallsâ€ by substantial reprogramming of root cell development. They usurp and modulate the plant cell cycle machinery for their benefit. RKN induced giant-feeding cells undergo acytokinetic mitosis and DNA endoreduplication and are surrounded by actively dividing neighboring cells. The cell cycle machinery is important to allow the induction and maintenance of the nematode feeding site development and certain core cell cycle genes have been shown to be crucial for nematode feeding site development. During the cell cycle progression, there are the presence of cell cycle checkpoint control among them WEE1. Arabidopsis WEE1 controls the cell cycle with major role in preventing cells from dividing until DNA is repaired and replication normalized. WEE1 is a nuclear kinase involved in terminal phosphorylation and inactivation of cyclin-dependent kinase 1-bound cyclin B, by inducing G2 cell cycle arrest in response to DNA damage. Our functional analysis using microscopy approaches revealed that lack of WEE1 protein will induce mitotic activity in galls, also cumulative mitotic defects possibly due to the lack of the appropriate timing for proper DNA replication and repair in mature giant cells. In addition, we have shown that stress inducing drug treatments on nematode infected roots, slows down mitosis in all gall cells most likely by inducing check point control. Finally, this study allowed us to gain insight into the possible role of this key cell cycle regulator during RKN feeding site development.

OC-28

Phylogeography of the vector nematode Xiphinema index using mitochondrial and microsatellite markers highlights its Eastern origin closely linked to grapevine domestication

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The economic impact of the dagger nematode Xiphinema index is high in Western vineyards by transmitting the damaging Grapevine fanleaf virus. Our phylogeographical study based on mitochondrial sequences and microsatellite loci used more than 80 X. index representative samples collected from the Middle- and Near East, the Eastern-, Central- and Western Mediterranean, and the Western countries (Europe and the Americas). In this mainly (meiotic) parthenogenetic species, the mitochondrial marker CytB was first considered for comparison of X. index with the related amphimictic vector species X. diversicaudatum. Xiphinema index exhibits a significantly lower intraspecific molecular variability than X. diversicaudatum, in agreement with the respective reproduction modes of both nematodes. We then showed that CytB, concatenated with additional mitochondrial genes ATP6, ND4 and CO1, display a robust phylogeographical pattern consisting in three clades grouping Eastern Mediterranean, Near- and Middle Eastern samples and a single clade grouping samples from Western Mediterranean, Europe and the Americas. The highest mitochondrial polymorphism is observed in one clade of Middle- and Near-East samples that overlaps the Transcaucasia and Southern Caspian Sea region from where grapevine has been presumably domesticated and that likely overlaps the nematode native area. East-to-west nematode dissemination appears to match that of its domesticated grapevine host during the Antiquity mainly by the Greeks and then the Romans. In Western Mediterranean, Europe and the Americas, two close and almost exclusive mitochondrial haplotypes are detected. The first haplotype, found in vineyards from the Southern Iberian Peninsula, Bordeaux and Provence, exhibits a high microsatellite polymorphism. By contrast the second haplotype contains a single predominant microsatellite genotype surprisingly widespread in most Western countries. This is almost certainly due to its recent dispersal during the massive grapevine replants following the 19th century phylloxera crisis. Our data provide an improved knowledge of X. index diversity for future pest control strategies.

OC-29

Conservation Biological Control: Impact of the various non-cropped components of landscape elements on phytophagous and natural enemies

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Pests cause increasing yearly losses to agriculture as well as plant production costs. Biological control appears to be a more environmentally friendly alternative to chemical control. We seletcted 11 non-crop plants to figure out whose impact on the fitness on natural enemies(Harmonia axyridis), natural enemies and pests diverstiy, yield of tomato both in lab experiements and field trials in the year of 2017 and 2018. The results showed that the longevity and fecundity of H.axyridis and tomato yield is significantly increased.

OC-30

Impact of pest functional types on plant-mediated indirect interactions

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Plants can be attacked subsequently and simultaneously by multiple functional types (feeding guilds) of pests in natural ecosystems and defensive responses can be induced to protect plants against the attackers (Soler et al. 2012). Attacks by multiple pests differentially activate plant defenses, which may lead to positive (i.e. facilitation), negative (i.e. antagonism), or no effects on the performance and preference of subsequently arriving pests (Ali & Agrawal 2014; Kroes et al. 2016). Depending on the functional types of attacking pests, variations in different phytohormone signaling pathways may lead to different regulations of plant defenses, which have further consequences for indirect interactions among these pests (Heidel & Baldwin 2004). In our study, we tested the plant-mediated interactions on life history traits of four differently functional types of pests, i.e., piercing-sucking pest (Macrosiphum euphorbiae), chewing pest (Helicoverpa armigera or Spodoptera exigua), plant pathogen (Oidium neolycopersici) and root-knot nematode (Meloidogyne incognita), on tomato plants, Solanum lycopersicum. Pests attacked plants subsequently or simultaneously and their species diversity from 1 pest to 4 pests was measured in our experiment to explore the indirect interactions. When the four different functional types of pests infested tomato plants at the different time, below-ground herbivores unidirectionally effect on above-ground pests. M.incognita had a negative effect on population growth of M.euphorbiae while it had a positive influence on O.neolycopersici. There was a strong interaction among nematodes, aphids and fungi. The treatment with the combination of M.incognita, M.euphorbiae and O.neolycopersici has the smallest aphid population, the largest fungal area and the least gall number of nematodes. Moreover, pest species diversity gradually decreased the population quantity of aphids and there was a linear correlation (R2 = 0.9564) between aphid population and numbers of pest species on a plant. However, when these four functional types of pests attacked tomato plants simultaneously, above-ground pests (M.euphorbiae and O.neolycopersici) have the positive influence on below-ground pest (M.incognita). In addition, S.exigua and O.neolycopersici significantly decreased aphid population. Furthermore, the weight increase of S.exigua was positively linear correlated (R2 = 0.9717) with pest species diversity on a tomato plant.

Ali JG, Agrawal AA (2014). Asymmetry of plant?mediated interactions between specialist aphids and caterpillars on two milkweeds. Functional Ecology, 28(6): 1404-1412. Heidel AJ, Baldwin IT. (2004). Microarray analysis of salicylic acid? and jasmonic acid?signalling in responses of nicotiana attenuata to attack by insects from multiple feeding guilds. Plant Cell & Environment, 27(11): 1362-1373. Kroes A, Stam,JM, David A, Boland W, van Loon JJ, Dicke M, Poleman EH. (2016). Plant-mediated interactions between two herbivores differentially affect a subsequently arriving third herbivore in populations of wild cabbage. Plant Biology, 18(6): 981-991. Soler R, Badenes-PÃ©rez FR, Broekgaarden C, Zheng SJ, David A, Boland W, Dicke M. (2012). Plant?mediated facilitation between a leaf?feeding and a phloem?feeding insect in a brassicaceous plant: from insect performance to gene transcription. Functional Ecology, 26(1): 156-166

OC-31

Effect of density and host distribution on the spatial diffusion of Trichogramma cacœciae.

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Tichogramma sp. are among the most studied biological control agents around the world and are used against a broad range of pest species. Nonetheless their use can still be improved and their behavior further understood. Specifically, the movement or dispersal of biological control agent is considered as one of the essential components for the success or failure in the field. The minute size of trichograms makes this particularly challenging to study. Most existing work addresses either very fine (behavioral) or fairly large (populational) spatial and temporal scales. We propose a novel experimental approach in laboratory conditions to understand the effects of population density, host presence and the spatial distribution of hosts on the diffusion and performance of Trichogramma cacÅ“ciae. We built an original experimental setting in which a 6-meter long linear arena is folded as a double spiral. By taking high-resolution pictures every minute and using dedicated image analysis methods, we could track individuals over several meters and eight hours, thus bridging the behavioral and populational scales. We showed that host presence had a positive impact on the diffusion capacity of trichograms regardless their spatial distribution. This result was not expected considering the significant time needed to parasitize a host. Furthermore, a higher initial density of individuals lead to a greater diffusion rate than a lower initial density, at least during the first four hours of the experiment. The presence of hosts in an environment could motivate individuals to keep searching during the whole experimental time. Without the hosts, we identified a diffusion threshold that could be explained by the lack of chemical or visual stimuli in an empty environment.

OC-32

The Range Pinning: When populations are frozen in space

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Spatial dynamics of expanding populations depend on extrinsic factors like habitat quality and quantity, or intrinsic factors related to population demography. In particular, low density on the expansion front may trigger what is known as Allee effects. In some cases, Allee effects may even cause a â€œpinningâ€ phenomenon, in which a population is unable to recolonize its suitable habitat after a local extinction.

Range pinning has never been confirmed empirically and its generality remains to establish.

We hypothesized that other demographic factors than Allee effects correlated with colonisation failures at small density could also create range pinning such as positive density-dependent dispersal. In order to confirm our hypothesis, we chose a joint approach of modelling and laboratory experiments.

We used individual based models to investigate the possibilities for range pinning in presence of Allee effects or of positive density-dependent dispersal. Then, we monitored recolonization dynamics following local extinctions in laboratory microcosms using Trichogramma hymenopteran wasps, a biological model that displays positive density-dependent dispersal.We evidenced range pinning in presence of positive density dependent dispersal in both simulations and experimental microcosms, provided that the carrying capacity of the habitat is low. This suggests that invasions could be managed by modulating intrinsic factors of populations or by modifying the landscape, such as a reducing carrying capacity. If such proposals had already been made for Allee effects, as far as we know, this is the first time that positive density dependent dispersion is put forward for this kind of management.

OC-33

The presence of secondary symbionts modulates phenoloxidase (PO) in the pea aphid and the host susceptibility to biotic and abiotic stress

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The pea aphid Acyrthosiphon pisum, a major pest in agronomy, harbors an obligatory symbiont, and can host eight different facultative symbionts. Its association with the obligatory symbiont Buchnera aphidicola, which occurred more than 100 million years ago, fulfills its nutritional needs and is essential for its reproduction. The facultative symbioses, more recent, are interesting insofar that they provide various extended phenotypes to the pea aphid, from adaptation to the host plant to resistance to abiotic (heat) or biotic (fungi, parasitoids) stress. Aphid immune components, notably immune cells, are also affected by the presence of facultative symbionts, depending on the symbiont and the symbiont strain. However, the insect immune defense is also based on a humoral component (i.e. antimicrobial peptides, phenol oxidase (PO)) and we therefore addressed the question of the effect of the presence of facultative symbionts on this component. Additionally, we observed the immune and ecological changes of aphid lines harboring different facultative symbionts under the abiotic and biotic stress. These results will participate in understanding the role of facultative symbionts in modulating the physiology and evolution of aphids.

OC-34

Phylogenetic study of Macrophage Migration Inhibitory Factor (MIF) cytokines and analysis of their role in host - parasite interactions

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MIF (Macrophage Migration Inhibitory Factor) crucial cytokines of the immune system of vertebrates. Some parasites, such as Filarial worms and ticks, secrete MIF proteins into their vertebrate host, where they modulate the immune response. Recently, we showed that a MIF protein is produced in the salivary glands of aphids and secreted during feeding. The protein interferes with the plant immune system and is required by aphids to establish an interaction with the host plant (Naessens et al., 2015). Plants, too, possess genes encoding MIF proteins (Panstruga et al., 2015), but their function is yet unknown.

The presence of MIF proteins in evolutionary distant organisms and their versatile roles in both host immunity and parasitism raise many questions about the evolution of this protein family. Here, we present a phylogenetic reconstruction of MIF proteins from animal and vegetal kingdoms. The evolutionary history of MIF is discussed with regards to the biology of species (parasitic or free living species) and suggest the existence of differential selection pressures across animal and vegetal phyla.

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OC-35

Cell composition of periodontal ligament extemporaneously just after dental extraction and after culture

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Introduction: The aim of this study is to quantify cell populations and especially mesenchymal stem cells (MSCs) present in the periodontal ligament (PDL) extemporaneously at the time of the dental extraction after enzymatic dissociation and to establish a kinetics to follow these populations in culture through different passages for the purpose of expansion and cell differentiation.

M & M: We collected 10 healthy teeth just after extraction from young adults aged 18 to 26 years old. After overnight storage in a recovery medium, PDL was separated from the root surface and an enzymatic dissociation (for 30 to 40 minutes at 37°c) was performed using a mixture of 3mg/mL collagenase type I and 4mg/mL of dispase in DMEM medium. Then, a flow cytometric analysis with a FACS Canto II was performed with MSCs specific markers: CD105, fibroblasts markers CD10 and CD29, and finally hematopoietic markers CD45. Subsequently, in an other set of experiments, these cells were cultured in 6-dishes containing a-MEM supplemented with antibiotics and serum (20%) at 37°C for 7 days.

Results: Dissociation allowed an average of 100,000 cells per PDL. The analysis in flow cytometry allowed us to determine that the PDL is composed on average of 33,7% ± 21,4% of fibroblast cells exhibiting the CD29 and CD10 markers and 38,7% ± 18,5 of the hematopoietic cells exhibiting CD45, but also of 2,5% ± 2,2% of Mesenchymal Stem Cells with CD105. MSCs markers were followed through culture and their population appears exponential as passages: from 2,5% of MSCs phenotype after collection, the culture permits to obtain 100% of MSC expressing this same marker after only 2 passages

Discussion: We therefore made several hypotheses: either the cultured MSCs proliferated to the detriment of the fibroblasts and the hematopoietic cells present mostly during the sampling, or the cultured fibroblastic cells acquired these specific markers for MSCs during culture.

Conclusion: Our results suggest that MSCs are present in small amounts in physiological PDL in vivo. After sampling and culturing, it is possible to obtain large amounts of MSCs which could then be used for therapeutic purposes, in particular for their immunomodulatory properties in periodontitis and peri-implantitis or for regenerative medicine.

OC-36

The role of KLF10 in the circadian coordination of liver homeostasis

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The circadian timing system (CTS) rhythmically controls most aspects of physiology and behaviour over the 24-hour day and perturbations of the CTS lead to metabolic disorders. We previously showed that the transcription factor KrÃ¼ppel-like factor 10 (KLF10) displays a circadian expression in liver. Using a systemic knockout model, we also established that KLF10 serves as a link between the core circadian clock and metabolism via the regulation of genes involved in lipid and glucose homeostasis. To better understand the role of KLF10 specifically in the liver, we generated a mouse-model with a conditional hepatocyte deletion of Klf10 (hK10) and analyzed the coding transcriptome of these mice around the circadian cycle. Results showed that the deletion of Klf10 in hepatocytes leads to loss or gain of rhythmicity of a significant number of transcripts without affecting expression of core clock genes. Phase set enrichment analysis indicated loss of temporal coordination of transcripts involved in glucose homeostasis, mitochondrial metabolism, proliferation and inflammation in hK10 mice. Interestingly, Klf10 deletion gives rise to de novo oscillations of transcripts that otherwise display low amplitude or â€“no rhythmicity in control mice. Furthermore, ~50% of these genes gain oscillation during a time window in which the expected peak expression of Klf10 normally occurs in wild type mice. Thus, as KLF10 has been previously described as a transcriptional repressor, this subset of genes may include direct targets of KLF10. Interestingly, among the genes upregulated during this time-window is Polo-like Kinase 3 (Plk3) which encodes a kinase regulating the p53, PIK3 and hypoxia signalling pathways which have all been previously involved in metabolism. This data provides new insights into how KLF10 aids in fine-tuning the coordination of the liver circadian transcriptome over the 24h day.

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Keywords: Circadian Clock, Liver Metabolism, Transcription Factor, Functional Genomics

OC-37

Noninvasive evaluation of non-alcoholic fatty liver disease by assessing liver function and serum markers

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Overweight and obesity dramatically increased in the last years. Hepatic complications of obesity, integrated in the term of non-alcoholic fatty liver disease (NAFLD), is a spectrum of abnormality ranging from steatosis to non-alcoholic steatohepatitis (NASH), potentially leading to cirrhosis. Despite its invasiveness, liver biopsy still remains the gold standard to diagnose and evaluate the stage of NAFLD. Henceforth, noninvasive approaches are required, easier and more secure. We here investigate two different and complementary approaches to non invasively diagnose NAFLD: (A) Indocyanine green clearance (ICG) test is performed since years to assess hepatic function before partial-hepatectomy, or after liver transplantation. In a prospective cohort study, we investigated morbidly obese individuals underwent bariatric surgery with scheduled hepatic biopsies. Liver biopsy specimens were classified according to the NAFLD activity score (NAS) : 6 (23.1%) patients revealed manifest NASH, and 5 patients were considered borderline (19.2%). A closed correlation was observed between ICG clearance test and hepatic steatosis (r = 0.43, p=0.03), NAS (r=0.44, p=0.025) and fibrosis (r = 0.49, p=0.01). In obese patient, we have shown that non-invasive evaluation of liver function with indocyanine green clearance test well correlated with histological features of NAFLD (1). (B) Composite scores including serum levels of hepatocyte apoptosis (caspase-generated cytokeratine 18 fragments, K18 fragments) associated with clinical and biological data could predict the presence of NAFLD as we previously reported (2). Since liver injury is a feature of NAFLD and is mainly mediated by hepatocyte apoptosis and necrosis, we here evaluated if the serum level of a pan-marker of hepatocyte death (total K18) could improve the accuracy of the diagnosis of NASH. In 362 morbidly obese patients, we shown that serum K18 alone had an area under the receiver operating characteristic (AUROC) curve = 0.80 to predict the presence of advanced NASH (severe steatosis and NAS?5). Among the different composite models generated, AUROC curve of the scoring system including K18 with ALT and waist circumference was 0.87 to predict the presence of advanced NASH. Possibility of the new composites models including the hepatic function test and serum K18 level to be an alternative to liver biopsy in the follow-up of obese patients and in the evaluation of treatments should be assessed.

(1) Danin PE et al. Non-invasive Evaluation of NAFLD with Indocyanine Green Clearance Test: a Preliminary Study in Morbidly Obese Patients Undergoing Bariatric Surgery. Obes Surg. 2018 Mar;28(3):735-742. (2) Anty R et al. A new composite model including metabolic syndrome, alanine aminotransferase and cytokeratin-18 for the diagnosis of non-alcoholic steatohepatitis in morbidly obese patients. Aliment Pharmacol Ther. 2010 Dec;32(11-12):1315-22.

OC-38

REDD1 deficiency protects from high fat diet induced metabolic diseases

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Obesity leads to the development of metabolic diseases including type 2 diabetes and nonalcoholic fatty liver disease. Hypoxia occurs within the adipose tissue and induces the expression of target genes such as REDD1, an inhibitor of mTORC1. We have shown that REDD1 is involved in insulin signaling pathway [1] and in the activation of inflammation [2]. Moreover, REDD1 expression is increased in adipose tissue and liver of obese mice. To study its implication in the development of obesity and its complications, wild-type (WT) and REDD1 knockout (KO) mice were subjected to high fat diet.

In standard diet, no differences of gain weight were observed. However, REDD1 KO mice display hyperphagia, higher locomotor activity, lower epidydimal adipose tissue mass and fasted glycemia compared to WT mice. Both WT and REDD1 KO mice had similar glucose tolerance.

Upon high fat diet challenge, the expression of REDD1 is increased in adipose tissue and liver. REDD1 KO mice gained less weight, but display the same insulin resistance and glucose intolerance than the WT mice. Interestingly, the liver injury evaluated by the serum levels of alanine-aminotransferase (ALT), liver weight, steatosis, hepatic triglycerides and lipogenesis as determined by FASN expression were decreased in REDD1 KO mice compared to WT mice.

In conclusion, absence of REDD1 seems to prevent the development of hepatic steatosis suggesting that REDD1 plays an important role in the development of complications of obesity.

[1] : Regazzetti et al. : PLoS One. 2012;7(12):e52154 ; [2] : Pastor et al. : Sci Rep. 2017 Aug 1;7(1):7023

OC-39

PLA2R1 as the major autoantigen in Membranous Nephropathy: from epitope spreading to personalized medicine.

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Primary membranous nephropathy (MN) is a rare but severe autoimmune kidney disease and the most common cause of nephrotic syndrome. The clinical evolution of MN is complex and treatment is controversial. There is a need for better biomarkers to identify patients at risk of severe disease and orient therapy. In 2009, the phospholipase A2 receptor (PLA2R1) was described as the major autoantigen in MN1, which led to a rapid development of diagnosis assays. Approximately 70% of patients have circulating autoantibodies targeting PLA2R1 at the podocyte surface, leading to in situ immune complex formation, podocyte injury and proteinuria. However, the biological function of PLA2R1 in human kidney is still unknown. PLA2R1 is a 180 kDa transmembrane glycoprotein consisting of 10 extracellular domains with multiple disulfide bonds. Three domains containing epitopes were identified: CR, C1 and C72. The severity of MN and poor outcome is likely associated with multiple antibodies produced by a mechanism of epitope spreading. However, their exact number and location are still unknown. Furthermore, there are controversial findings concerning the CR and C1 domains which may assemble to form a single overlapping immunodominant epitope within the CR-FnII-C1 region3 or may be independent epitopes2. Our aim was to clarify the number of domains containing epitopes in the proximal and distal regions of PLA2R1. To clarify the conundrum between CR and C1 domains, we prepared and expressed in HEK293 cells a series of CR-FnII-C1 triple domains with protease cleavage sites inserted between CR-FnII or FnII-C1. Reactivities of these domains were tested with different patientsâ€™ sera before and after protease cleavage. Before cleavage, all sera reacted against the triple domain proteins. After cleavage, some sera had reactivities limited to CR or C1 while others reacted with both CR and C1 as isolated domains, demonstrating that there are two independent epitopes. In addition, we prepared and expressed in HEK293 cells several recombinant proteins with overlapping domains within the C-terminal region of PLA2R1 and tested by ELISA the reactivity of a cohort of patientsâ€™ sera. This screening led us to identify C5 and C8 as new epitope-containing domains. The clinical application of the 5 PLA2R1 domains containing epitopes in disease activity is currently being analyzed and this work will help to design new diagnosis and prognosis tools towards MN personalized medicine.

1. Beck et al, NEJM 2009. 2. Seitz-Polski&Dolla, JASN, 2016. 3. Kao et al, JASN, 2015

OC-40

Novel ELISA for Thrombospondin type 1 domain-containing 7A autoantibodies in membranous nephropathy

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Membranous nephropathy (MN) is a rare autoimmune kidney disease with an incidence of 1.3/100,000 yet it is a leading cause of nephrotic syndrome in adults. It is characterized by the accumulation of immune deposits in the glomerular capillary walls. The outcome of the disease varies from spontaneous remission to end stage kidney disease, with high proteinuria. Considerable advances have been made in the understanding of the pathophysiology of MN with the recent identification of 1) PLA2R1 as the major autoantigen for about 70% of patients1 and 2) THSD7A as a second autoantigen for another group for about 2-5% of patients2. Both autoantigens are expressed in human podocytes and are membrane proteins (180 and 250 kDa, respectively) with a long extracellular region comprising multiple but distinct domains with disulfide bonds. Interestingly, both PLA2R1 and THSD7A autoantibodies bind to conformational epitopes present in one or more respective domains and are predominantly of the IgG4 subclass.

Detection of PLA2R1 and THSD7A autoantibodies and accurate measurement of their titers are becoming important biomarkers to diagnose MN and predict outcome. Various assays are available for diagnosis and measurement of circulating PLA2R1 antibodies in serum including a commercial ELISA and immunofluorescent test (IIFT) test in addition to western blot (WB). In contrast, detection of THSD7A autoantibodies is only possible by WB and IIFT , allowing a semi-quantitative assessment of autoantibody levels in serum. Developing a more robust and rapid assay for the sensitive and quantitative measurement of antibody levels in THSD7A-associated MN patients would be helpful for both diagnosis and clinical follow-up. Here, we describe the set-up of the first ELISA for the quantitative detection of anti-THSD7A antibodies. We used the assay to screen a combined cohort of 1012 MN patients and identified 28 THSD7A-positive patients, indicating a prevalence of 3%. We further described the clinical characteristics of this population for age, gender, disease activity and possible links to etiology including malignancy. Our data highlights a possible link between MN and pregnancy and suggests a poor association between MN and malignancy in our population of THSD7A-positive patients. The novel ELISA test correlates significantly with the commercially available IIFT and can be used to identify patients with THSD7A-associated MN and monitor antibodies during follow-up.

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OC-41

Analysis of SOX11 function in early and late mouse kidney development

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Sox11 is a member of SoxC family of transcription factors, which plays an important role in the development of various organs in vertebrates. Sox11 knockout mice die soon after birth and display a wide variety of renal anomalies, including duplex kidneys and nephrogenesis defects. In this project, we analyzed the renal phenotype of Sox11 knockout mice and discovered that the duplex kidneys are caused by a rostrally extended domain of metanephric mesenchyme (MM). To study nephrogenesis defects in vitro, we set up a renal progenitor cell culture system, which allows for the timed deletion of SoxC genes using a tamoxifen induced knockout. Cells lacking SoxC genes were found to display reduced expression in transcription factor Tead2, coupled with increased expression of its targets: Serpine1 and Cyr61. To validate these findings, and discover the direct downstream targets of SoxC genes, we developed a system for ChIP-seq analysis of embryonic cap mesenchymal cells. Data acquired in these experiments will be used to construct a model for the action of SOXC proteins during the process of nephrogenesis.

OC-42

The tyrosine phosphorylated prosurvival form of Fas intensifies the EGF-induced signal in colorectal cancer cells through the nuclear EGFR/STAT3-mediated pathway

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Tyrosine phosphorylation of Fas (TNFRSF6/CD95) in its death domain turns off Fas-mediated apoptosis, turns on the prosurvival signal, and has implications in different cancers types. We show here that Fas in its prosurvival state, phosphorylated at Y291 (pY291-Fas), functionally interacts with the epidermal growth factor receptor (EGFR), a key cancer-driving protein and major therapeutic target. Using an evolution-guided pY291-Fas proxy, RNA interference, and site-specific phospho-protein detection, we show that pY291-Fas significantly intensifies EGFR signaling in anti-EGFR-resistant colorectal cancer cells via the Yes-1/STAT3-mediated pathway. The pY291-Fas is essential for the EGF-induced formation of the Fas-mediated EGFR/STAT3 signaling complex consisting of Fas, EGFR, Yes-1, Src, and STAT3. The pY291- Fas,which also accumulates in the nucleus in a complex with EGFR and STAT3 upon EGF treatment, plays an active role in promoting the nuclear localization of phospho-EGFR and phospho-STAT3, the expression of cyclin D1, the activation of STAT3-mediated Akt and MAPK pathways, and cell proliferation and migration. This novel cancer-promoting function of phosphorylated Fas in the nuclear EGFR signaling constitutes the foundation for developing prosurvival-Fas targeted anti-cancer therapies to overcome disease recurrence in patients with anti-EGFR resistant cancer.

OC-43

Dissection of the role of the E3 ubiquitin ligase LNX2 in the control of Fas cell death signaling: potential applications in preventing colorectal cancer recurrence.

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Colorectal cancer (CRC) is the third commonest malignancy worldwide. The disequilibrium between cell death and survival signals due to defective programmed cell death is a key factor in carcinogenesis. Fas (CD95, TNFRSF6), a member of the TNF receptor family, is amongst the best characterized apoptosis inducer. The apoptotic signaling induced by Fas, upon activation by its ligand (FasL) initiates caspase activation leading ultimately to cell destruction. The death receptor Fas activation can induce both death and non-death signaling pathways. The main aim of my Laboratory is to understand when and how one role of Fas is favored over the other. In this context, we recently demonstrate that establishment of cell-cell contacts and cell polarity inhibits the pro-death signaling of Fas. We demonstrated that pro-apoptotic signaling of Fas is inhibited by two mechanisms: 1) Fas is sequestered in E-cadherin dependent cell-cell junction, which impairs efficient FasL binding; 2) at junction, Fas interacts directly with the polarity molecule Dlg1. We demonstrate that association of Dlg1 with the PDZ domain of Fas located in its C-terminal region strongly inhibits Fas cell death signaling, possibly by interfering with the formation of the cell death complex on Fas cytoplasmic domain.

In order to understand further the role of the PDZ domain of Fas in the regulation of its signaling, a peptide pool-down experiment using the C-terminal region of Fas followed by proteomic analysis was done to identify other possible Fas molecular partners. Among the new proteins identified, we focus our interest on LNX2, a RING E3 ubiquitin ligase with PDZ domain. LNX2 has been characterized as an inhibitor of apopotosis and found overexpressed in CRC. To determine whether anti-apoptotic effect of LNX2 could be mediated by its role on Fas signaling, we performed loss of function and overexpression experiments of LNX2 in two CRC cell lines. We were able to show that LNX2 exerts an inhibitory role on Fas-mediated cell death signaling by regulating Fas receptor trafficking. We were also able to demonstrate that LNX2 interacts not only with Fas but also with Dlg1. Our study reveals a new role of the E3 ubiquitin ligase LNX2 in regulating Fas cell death signaling in CRC cell line. Further study will be necessary to understand the exact molecular mechanism by which LNX2 modulates Fas receptor trafficking.

OC-44

PGC-1a controls an onco-metabolic program to limit prostate cancer aggressiveness

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Prostate cancer (PCa) is the third cause of cancer death in men and deaths are due to advanced metastatic PCa. Metabolic reprogramming has been shown to play a major role in cancer aggressiveness; however, the metabolic pathways implicated in the formation of metastasis are poorly understood. In this context, we chose to study peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a) which plays a major role in cell metabolism and more specifically in the regulation of oxidative phosphorylation. PGC-1a is a master regulator of mitochondrial biogenesis and a recent paper suggests that low levels of PGC-1a may be associated with a poor prognosis in PCa. Then, we decided to study the role of PGC-1a on PCa aggressiveness and metabolism. We performed a knockdown (KD) of PGC-1a in prostate cancer cell lines using different shRNA. PGC-1a KD enhanced PCa cell proliferation, migration and invasion of LNCaP and DU145 cells. Conversely, overexpression of PGC-1a decreased cell migration. To determine the molecular mechanism implicated in this phenotype, we analyzed the expression of several genes controlling oncogenesis and metabolism. We found that c-myc and some other genes implicated in glutaminolysis were up regulated in PGC-1a KD cells. We then performed proliferation and transwell migration assay using c-myc inhibitors. These inhibitors reversed the pro-proliferative and the pro-migratory effects induced by the downregulation of PGC-1a. To characterize the metabolic modifications modulated by PGC-1a, we performed a steady-state metabolomic analysis. We demonstrated that the polyamine pathway (putrescine, spermine) is significantly up-regulated in cells where PGC-1a is downregulated. In accordance, the ornithine decarboxylase (ODC), a rate limiting enzyme of this pathway controlled by c-myc, is up-regulated in PGC-1a KD cells. We then decided to inhibit ODC with DFMO (?-difluoromÃ©thylornithine) and performed migration assay. We showed that the pro-migratory effects of PGC-1a KD cells are blocked by DFMO. Finally, in accordance with the results presented here, we demonstrated that the expression of PGC-1a is significantly downregulated in PCa patients and logically, c-myc and ODC are up regulated. Altogether, our results demonstrate that the downregulation of PGC1a increased c-myc expression and up-regulated polyamine synthesis. These onco-metabolic modifications are directly implicated in PCa aggressiveness.

OC-45

Fractalkine (FKNs) within a multi-modal therapy protocol of NSCLC bone metastasis : pre-clinical study in a murine model.

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Introduction : The anti-tumoral activity of soluble FKNs on the development of experimental bone metastasis of epidermoïd lung cancer in mouse was proved by previous research. This chemokine, the FKNs, when delivered locally, allow to increase the recruitment of intra-tumor leucocyte, and to disrupt the vicious cycle linking tumor proliferation and bone resorption. Our previous research could identify interesting variation of the level of expression of immune check-point (ICP) in mouse tumor treated be FKNs. We wanted to assess if the association of local delivery of FKNs and systemic administration of immunogenic chemotherapy or ICP inhibitors could increase the anti-tumor activity. Material and methods: We tested the efficacy of the association of locally delivered FKNs and systemic immunogenic chemotherapy (docetaxel and oxaliplatin) or systemic ICP inhibitor (anti PD1, anti CTLA4, anti TIM3) in a syngeneic model of experimental bone metastasis of epidermoïd lung cancer. The mice were treated with different association of those treatments at various moments of the tumor development. We are assessing: tumor weight at the time of sacrifice and a panel of gene's expression through qPCR to approach mechanisms. Results: The tumor weight was decreased of 76.8% after treatment by the association FKNs/ chemotherapy (p=0,006) VS 34% after chemotherapy alone and 53% after FKNs alone. This effect is associated with a modification of immune checkpoint gene's expression and with an increase of intratumoral leucocyte density. For the association of FKNs and immune check-point inhibitor, our work is still in progress. We noticed no effect or paradoxical effect on tumor weight with the adjunction of immune-check-point inhibitor to FKNs. We are currently processing the sample for qPCR in order to understand those macroscopic results. Conclusion: In progress…

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Chemotherapeutic stress triggers a protective horizontal mitochondrial transfer from mesenchymal stromal cells to AML leukemic blasts

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In a co-culture system we demonstrated that human Acute Myelogenous Leukemia (AML) cells uptake intact active mitochondria from bone marrow stromal cells. We evaluated that AML cells could increased their mitochondrial mass up to 14%, to elevate their mitochondrial ATP production by 1.5-fold. This transfer was found to require cell-cell contacts, to be unidirectional and was observed in vivo. It was greater in AML blasts compared to normal cord blood CD34+ cells. Interestingly, the transfer was enhanced by several chemotherapeutic drugs and stressful conditions such as hypoxia and glucose starvation suggesting an adaptative response to stress and inflammatory conditions triggered by chemotherapy. Mitochondria-receiving cells appeared less sensitive to mitochondrial depolarization after chemotherapy and displayed a survival advantage. This new mechanism involved in Environment-mediated drug resistance (EM-DR) could represent a future therapeutical adjuvant opportunity for AML treatment.

Moschoi et al. Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. Blood. 2016 Jul 14;128(2):253-64.

OC-47

Loss of EFA6-B, an EMT regulator, facilitates breast cancer development in vivo

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In breast cancer, 90% of death cases are due to aggressive metastatic tumors. The epithelial-mesenchymal transition (EMT) is believed to be a key process to mediate metastasis. One of the earliest events of EMT is the dissolution of the tight junction and the loss of cell polarity, two features essential to maintain epithelial functions (1, 2). We observed that EFA6B, a guanine nucleotide exchange factor for the small G protein Arf6, helps assemble and stabilize tight junctions and is required to maintain epithelial apico-basal polarity and phenotypic characteristics in mammary cells (3, 4). Next, using human breast cancer cell lines, we investigated the role of EFA6B (PSD4) in breast cancer initiation and progression. Organotypic 3D cell cultures, flow cytometry and in vitro functional assays highlighted the engagement into EMT and the acquisition of tumorigenic properties of cells downregulated for EFA6B. Orthotopic xenografts showed that MCF7 shEFA6B tumors grew twice as fast as their wild-type counterparts indicating that downregulation of EFA6B is causal to the increase of MCF7 cells tumorigenicity. In agreement, the analysis of EFA6B expression and histoclinical features of tumors from a cohort of 5252 breast cancer patients associated low levels of EFA6B with the aggressive triple-negative and claudin-low breast cancer subtypes.

Finally, transcriptomic analysis of the MCF7 shEFA6B cells revealed some differentially regulated proteins and enzymes of the extra-cellular matrix as well as cell-cell adhesion molecules that will help us decipher the mechanism by which EFA6B acts.

Our results identified EFA6B as a novel antagonist in breast cancer and they point to its regulatory and signaling pathways as potential therapeutic targets in aggressive forms of this disease.

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OC-48

Discoidin Domain Receptors DDR1 and DDR2 promote matrix-mediated drug resistance (MM-DR) to MAPK-targeting therapies in BRAF mutated melanoma

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In tumors, fibroblasts are primary producers of extracellular matrix (ECM), a dynamic network of structural and regulatory proteins that is constantly remodeled during tumorigenesis. The tumor-ECM crosstalk plays a fundamental role in malignancy regulating most if not all hallmarks of cancer such as invasion, survival and drug response. Targeted therapies against the BRAF oncogenic pathway have achieved limited clinical benefit to date in patients with melanoma due to development of acquired drug resistance. We reasoned that ECM biochemical and mechanical properties might determine melanoma cells responsiveness to targeted therapies.

To test this, we generated fibroblast-derived ECM from different fibroblasts isolated from the skin, lymph nodes or metastatic biopsies (melanoma-associated fibroblasts, MAF) and analyzed their ability to protect melanoma cells from MAPK inhibitor therapy. The composition, structure and rigidity were characterized by MS/MS analysis, collagen staining and atomic force microcopy, respectively.

Here, we find that both biochemical and mechanical properties of experimentally derived matrix differed remarkably depending on origin of fibroblasts. Adhesion of melanoma cells to the different ECM abrogates anti-proliferative responses to MAPK inhibition by maintaining phosphorylation of Rb, cell cycle progression, and expression of the pro-survival protein Survivin. Further, we observe that drug protective efficacy is variable in the different ECM settings with ECM-derived from lymphatic fibroblasts or MAF being particularly prone to resistance. MM-DR is dependent on tyrosine kinase receptors DDR1 and DDR2, two receptors for collagens. Genetic depletion of DDRs or their targeting by imatinib overcome MM-DR in vitro and in vivo, and markedly reduces targeted therapy-induced tumor fibrosis in melanoma xenografts.

Our data reveal an unexpected role of DDRs in resistance and bring insights into how ECM modulates tumor cell behavior in metastatic resistant niches. Targeting DDRs with imatinib/Gleevec, a clinically approved drug for leukemia may be beneficial for patients with melanoma on targeted therapies.

OC-49

Inhibition of the IRE1? branch enhances the anti-tumoral activity of Sorafenib in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is an aggressive cancer associated with a very poor prognosis: itâ€™s the second cause of death by cancer. Moreover, clinical treatment of HCC remains challenging because of a lack of effective chemotherapy. Sorafenib remains the only drug approved for the treatment of HCC.

The pathophysiological mechanisms underlying the etiology of HCC remain poorly understood. Most cases of HCC are associated with chronic liver diseases. Understanding these mechanisms and identifying potential therapeutic targets is of utmost importance.

Endoplasmique Reticulum (ER) stress was shown to be involved in the progression and the developpment of this pathology. We postulated that Bax Inhibitor 1 (BI-1), an inhibitor of the most conserved endoplasmic reticulum sensor IRE1?, could play a central role in the pathogenesis of HCC.

Recently, we have established a link between the activation of the IRE1? arm and the NLRP3 inflammasome in steatosis-steatohepatitis transition in humans and animals models.

Our results indicate a strong reduction of Bi-1 expression (80%) associated with an increase of the IRE1? pathway that correlates with enhanced inflammasome activation in several human hepatoma cells lines compared to non-tumorous primary human hepatocytes.

The pharmacological inhibition of the IRE1? pathway accentuates the pro-apoptotic and anti-angiogenic effect of Sorafenib as well as reduces inflammasome activation.

News small molecules synergize with Sorafenib, potentiating the induction of cell death (apoptosis and necrosis), the reduction of tumor proliferation and migration in vitro and reduce tumors size in xenograft models.

In conclusion, this study demonstrates that the sur-activation of IRE1? and inflammasome are involved in the pathogenesis of HCC. Targeting IRE1? would represent a potential therapeutic strategy in the treatment of HCC.

OC-50

Matrix stiffness contributes to the chemoresistance of head and neck squamous cell carcinoma to EGFR inhibitors

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IRCAN

My research aims at identifying the molecular processes of EGFR targeted therapy resistance trigger by the tumor niche in head and neck squamous cell carcinoma (HNSCC). Despite the fact that over 15% of HNSCC overexpress EGFR (epidermal Growth Factor Receptor), HNSCC are refractory to EGFR TKIs (Tyrosine Kinase Inhibitors) targeted therapy and yet the molecular and cellular mechanisms of EGFR-TKIs resistance in this HNSCC are unknown. The tumor niche plays an important role in conventional chemotherapeutic resistance. Cancer associated fibroblasts (CAFs), the most prominent stromal cell in tumor niche, participate in this process. Notably, CAFs are responsible for tumor tissue fibrosis an excessive extracellular matrix (ECM) remodeling that increases matrix stiffness. In carcinoma cells, adhesion to stiff substrate triggers mechano-dependent intracellular signaling pathways that favor tumor resistance to conventional chemotherapies. My work demonstrates that stiff ECM substrate has high impact on overall survival of cancer cells by 60% more survival of cells plated on stiff matrices compared to soft, when treated by the gefitinib EGFR TKI. Same effect was observed on matrix derived from CAFs that is known to be stiffer compared to the one derived from fibroblasts isolated from normal skin. SiRNA screening of 52 ECM components identified by mass spectrometry analysis of CAF and fibroblasts derived ECM and RNA sequencing of SCC12 cells plated on soft and stiff matrix revealed Finbulin 2 and TNFAP3/A20, respectively, as main drivers of EGFR TKI resistance in HNSCC. Here, we aim at demonstrating that targeting Finbulin 2 in derived matrix and/or TNFAP3 in SCC cells lying on stiff matrices, revert the EGFR TKI resistance triggers by the tumor niche. Our overall goal is to identify novel therapeutic targets environment with reduced resistance opportunity.

OC-51

Role and regulation of ASAH1 in melanoma

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Melanoma is the most deadly form of skin cancer, it derives from melanocytes that are responsible of skin pigmentation. At the metastatic stage, melanoma prognosis is very poor. Indeed, even if targeted therapies and immunotherapies were developed the last few years, more than 50% of patients are unresponsive or become resistant to these treatments. Therefore, it is necessary to keep understanding the molecular mechanisms involved in melanomagenesis to find better therapeutic strategies.

Several lines of evidence indicate that cancer cells show alterations in metabolic pathways. In melanoma, we've just only begun to define these metabolic pathways and the players. Interestingly, a bioinformatic study revealed that the acid ceramidase ASAH1 (N-AcylsphingosineAmidohydrolase 1) showed the highest expression in melanoma cells compared to other tumor cells. Moreover, analysis of publicly available datasets indicated that high ASAH1 expression is associated with a poor prognosis. ASAH1 has a central role in sphingolipid metabolism by controlling the conversion of ceramide into sphingosine. We therefore examined the regulation and role of ASAH1 in melanoma cells.

Our results indicate that ASAH1 is highly expressed and active in about 60% of melanoma cells and that its expression is regulated by MIcrophthalmia-associated Transcription Factor (MITF), a transcription factor critical to melanocyte development, functioning and transformation, thereby identifying ASAH1 as a new MITF target gene. Further, our findings demonstrate for the first time the involvement of MITF in the control of sphingolipid metabolism. We are currently uncovering the way ASAH1 exerts its effects to potentially offer new targets for metastatic melanoma treatment.

Poster presentation
Abstract

P-1

The nitrogen-fixing symbiosis : role of bacterial VapBC toxin antitoxin modules.

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The symbiotic interaction between the bacterial species Sinorhizobium meliloti and the leguminous plant Medicago truncatula leads to the formation of a new root organ, the nodule, where differentiated bacteria convert the atmospheric nitrogen (N2) in ammonium (NH4+). To better understand the intracellular lifestyle adaptation of bacteria, we study the role of the S. meliloti VapBC Toxin-Antitoxin (TA) systems during symbiosis. 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, allowing a post-transcriptional regulation due to its RNase site-specific activity.

Such modules were described in human pathogenic interaction with roles (intracellular survey) and environments (acidic pH, microoxy) consistent with those encountered in the vegetal symbiotic interaction.

A characterization of the 11 putative VapBC systems of S. meliloti encoded by the bacterial chromosome has been undertaken, in order to analyze their symbiotic role. The functional validation of these systems allowed to define the toxic activity of all VapC proteins when sur-expressed in Escherichia coli; demonstration of the mitigation effect by VapB has been initiated.

Infection of M. truncatula with bacterial mutants deficient in the VapC toxin component of VapBC modules, has shown 6 vapC toxin mutants among 11 have different altered symbiotic phenotypes: defect in infection, early or delayed nodule senescence. To determine precisely the key steps of the symbiosis impacted by the different toxin deficient mutants, molecular and cellular approaches have been realized.

At the molecular level, we have demonstrated that the VapC-7 protein of the VapBC-7 module, important for plant host viability, acted as a ribonuclease. To further correlate the toxin activity to a defined biological function during symbiotic interaction, the RNA precisely targeted by VapC toxins of biological importance will be identified. Indeed, due to the different phenotypes observed, some modules seem to promote fundamental biological functions involved in key steps of symbiosis.

These studies will provide knowledge about the bacterial partner and its involvement throughout a symbiotic interaction. In agronomy, this could offer the opportunity to improve efficiency and adaptation of the symbiotic interaction in the field.

P-2

Involvement of Kir2.1 in the BMP pathway maintenance during osteoblastogenesis

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Andersen's syndrome (AS) is a rare and complex disorder with skeletal muscle, cardiac muscle, and bone defects. AS is associated with heterozygous mutations of KCNJ2 that lead to a loss of function of the inwardly rectifying K+ channel : Kir2.1. Kir2.1 channels play a key role in maintaining the resting membrane potential in excitable tissues, and ensures the late repolarization phase of the cardiac action potential. While the function of the Kir2.1 channel can be easier to explain in excitable cells, its role in bone development remains to be elucidated.

We have generated human induced pluripotent stem (iPS) cells from healthy and AS patient muscular biopsies and have shown that this can be a good model for AS disorder. These cells were used to investigate the impact of Kir2.1 loss of function during osteoblast differentiation.

We show that loss of Kir2.1 channel function down regulates master genes expression during the osteoblast differentiation, leading to defective osteoblasts. This down regulation is due to a disruption of the Bone Morphogenetic Proteins (BMP) signaling pathway.

Our work reveals how the electrogenic activity of Kir2.1 K+ channels at the cell surface can control major intracellular signaling.

P-3

Deciphering molecular and cellular pathways in pain control: nature as inspiration for the rational design of new potent analgesics

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1. Affiliation nÂ°1, 2. Affiliation nÂ°2, etc

Buruli Ulcer represents the third most common mycobacterial disease after tuberculosis and leprosy. One of the most surpring features of this skin infection is the absence of pain in the early stages of the disease. Previously we showed that it is due to a potassium-dependent hyperpolarization of neurons triggered by Mycolactone, the main virulence factor of the bacterium responsible of the ulcer.

In this study, we revisit the signaling pathway triggered by Mycolactone on Angiotensin II type Receptor (AT2R) to activate TRAAK, a K2P potassium channel, to inhibit pain

P-4

GAPDH overexpression in the T cell lineage promotes angioimmunoblastic T cell lymphoma through an NF-B dependent mechanism GAPDH overexpression in the T cell lineage promotes angioimmunoblastic T cell lymphoma through an NF-B dependent mechanism

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key glycolytic enzyme is emerging as a key player in T cell function and cancer. We hypothesized that overexpression of GAPDH in the T cell lineage might reveal its role in T cell differentiation and malignancy. Therefore, we generated a mouse model overexpressing GAPDH exclusively in the T cell lineage (under control of the T cell specific promoter Lck, named pLck-GAPDH mice). Though no obvious phenotypic changes were detected in these mice at 6 weeks or 6 months, at later age (> 18 months) clear phenotypic changes were detected: 1) a generalized lymphadenopathy 2) splenomegaly with a greatly altered spleen architecture, 3) lymphocyte infiltrations in the liver and bone marrow. Detailed immune-phenotying showed that the tumor mouse tissues (spleen, liver, lymph nodes) were marked by a specific increase of CD4+PD1high CXCR5+ T follicular helper (Tfh) cells as compared to wt. These Tfh cells were strongly proliferating and clonal, confirming true lymphoma development. Moreover, Tfh cells were associated with increased levels of CD95+ GL-7+ germinal center B cells and inflammatory cytokine release. Gene expression

analysis confirmed that our murine lymphoma was equivalent to human angio-immunoblastic T-cell lymphoma (AITL), a rare aggressive cancer. Mechanistically, GAPDH induced NFkB pathway in our murine AITL mouse model as in patients. Remarkably, in vivo targeting of this pathway through inhibition of NIK, a key player in the non-canonical NF-KB pathway, combined with anti-PD1 immunotherapy resulted in increased survival of up to 70%. Moreover, we confirmed that this combination therapy reactivated CD8 anti-cancer immune response. In conclusion, GAPDH overexpression in T-cells resulted in valid preclinical model of AITL for evaluation of novel treatment regimens.

P-5

Impact of phospholipid polyunsaturation and asymmetry on membranes as deduced from molecular dynamics simulations

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Cellular membranes provide a balance between a protective barrier and an exchange interface. Phospholipids (PLs) are the major components of cellular membranes. They consist in a polar head group, a glycerol backbone, and two hydrophobic fatty acyl chains wherein the number of double bonds between the carbon atoms is variable. Most cellular membranes contain PLs with saturated and monounsaturated acyl chains. However, PLs with polyunsaturated omega 3 or omega 6 acyl chains are extremely abundant in a few specific membranes such as synaptic vesicles (Antonny et al., 2015). These variations in the fatty acyl profile of PLs suggest that polyunsaturated PLs confer specific physicochemical properties to membranes. For example, it has been shown that polyunsaturated PLs facilitate membrane deformation (Pinot et al., 2014). Here, we present a comprehensive analysis of the effect of fatty acyl chain unsaturation at position sn1 and sn2 in phospholipids on model membranes using molecular dynamics simulations. We show the great flexibility of polyunsaturated acyl chains and their impact on membrane properties such as thickness and permeability. A membrane composed of asymmetric polyunsaturated PLs is more malleable than a membrane composed of saturated or monounsaturated PLs but remains quite impermeable to small solutes. In contrast, PLs with two polyunsaturated chains form extremely disordered membranes, which lose their protective barrier character despite being readily deformable. This study suggests a rationale for the enrichment in asymmetric saturated/polyunsaturated PLs observed in some specialized organelles or cells.

Pinot, M., Vanni, S., Pagnotta, S., Lacas-Gervais, S., Payet, L.-A., Ferreira, T., Gautier, R., Goud, B., Antonny, B., and Barelli, H. (2014). Lipid cell biology. Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins. Science 345, 693–697

P-6

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 with their host plants. 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 effectorproteins 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 localization signals. MiEFF18 localized 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 a spliceosomal protein Sm 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 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.

Rutter et al, 2014. Mol Plant Microbe Interact. Sep;27(9):965-74. Nguyen et al, 2017. New Phytol. Jan;217(2):687-699.

P-7

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.

Benedito, V. A., Torres?Jerez, I., Murray, J. D., Andriankaja, A., Allen, S., Kakar, K., . . . Ott, T. (2008). A gene expression atlas of the model legume Medicago truncatula. The Plant Journal, 55(3), 504-513. De Zélicourt, A., Diet, A., Marion, J., Laffont, C., Ariel, F., Moison, M., . . . Frugier, F. (2012). Dual involvement of a Medicago truncatula NAC transcription factor in root abiotic stress response and symbiotic nodule senescence. The Plant Journal, 70(2), 220-230. Perez Guerra, J. C., Coussens, G., De Keyser, A., De Rycke, R., De Bodt, S., Van De Velde, W., . . . Holsters, M. (2010). Comparison of developmental and stress-induced nodule senescence in Medicago truncatula. Plant Physiol, 152(3), 1574-1584. doi:10.1104/pp.109.151399 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. Van de Velde, W., Guerra, J. C. P., De Keyser, A., De Rycke, R., Rombauts, S., Maunoury, N., . . . Goormachtig, S. (2006). Aging in legume symbiosis. A molecular view on nodule senescence in Medicago truncatula. Plant Physiology, 141(2), 711-720. Young, N. D., & Udvardi, M. (2009). Translating Medicago truncatula genomics to crop legumes. Current opinion in plant biology, 12(2), 193-201. Sheokand, S., Dahiya, P., Vincent, J., & Brewin, N. (2005). Modified expression of cysteine protease affects seed germination, vegetative growth and nodule development in transgenic lines of Medicago truncatula. Plant Science, 169(5), 966-975.

P-8

Characterization of bone extracellular matrix produced by RECQL4-deficient osteoblast

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Bone is a complex and mineralized tissue under permanent remodeling. The main cellular actors of bone remodeling are osteoblasts (OB), that synthetise the bone mineral matrix, osteoclasts (OCL) in charge of bone resorption, and osteocytes (OST) that act as mechanosensors. The bone extracellular matrix (ECM) is a dynamic network of molecules secreted by OB and OST that in turn regulate the behavior of all bone cells by modulating their proliferation, differentiation and function. As an example, senescent OB create a defective microenvironment through an altered secretome, which in turn stimulates OCL function. Meanwhile, another study shows that exposure to a decellularized ECM deposited by young OB rescues replication potential of aged OB precursors. Taken together, these observations highlight the connection between OB secretome and bone cells behaviour.

Our laboratory is studying two murine models of bone loss potentially associated with altered OB secretome. Aiming to decipher the mechanisms underlying bone loss in our models, we planned to investigate the relatonship between the OB-produced bone matrix and bone cells.

Here, we describe the methodologies used to produce, decellularize and characterize bone matrices synthesized in vitro by primary OB isolated from Recql4-deficient mice. recql4-/- mice exhibit a premature bone aging phenotype reminiscent of the one observed in patients harboring RECQL4 mutations. RECQL4 is a DNA helicase involved in genomic stability and its dysfunction has been associated with cellular senescence.

We first observed that bone matrix produced by recql4-/- OB are less mineralized than those from recql4+/- control OB. We next analyzed protein and mineral composition, ultrastructure and stiffness of the resulting matrices using different approaches such as proteomic analysis, scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX), SEM and atomic force microscopy (AFM). Our preliminary results will be presented and discussed.

We expect that the protocols we developped to analyze mineralized matrices will help to understand bone cells behavior in pathophysiological conditions such as premature bone ageing.

P-9

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. 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. 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. Probands were diagnosed with EOS, which in some cases was combined with other phenotypes such as ASDs or ID. Additionally, two single cases of patients

diagnosed with schizophrenia were analyzed in the course of this study. 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 gene STK33 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. The other candidate gene is INPP5A that mobilizes intracellular calcium and acts as a second messenger mediating cell responses to various stimuli. 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 genes. For instance, for STK33 we created a cellular model in the SH-SY5Y cell line by the CRISPR-Cas9 technique mimicking the potential mutation found within the patient. To describe the phenotype of the mutated cell line, we are performing the analysis of gene and protein expression as well as calcium imaging experiments and MEA (Multielectrode Array). For the other genes that have their fly homolog, we are generating a model in Drosophila melanogaster whose power as a model in the study of human genetic diseases has been widely validated by several studies.

P-10

Characterization of Individual Domains of the Arabidopsis Receptor-Like Kinase IOS1

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Oomycetes are devastating filamentous pathogens that impact ecosystems and agriculture. Our research aims at characterizing the molecular mechanisms that govern the establishment of disease in host plants. 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 the Arabidopsis receptor-like kinase "Impaired Oomycete Susceptibility 1" (IOS1), which downregulates abscisic acid (ABA) signaling and contributes to the infection success of filamentous, biotrophic pathogens such as oomycetes and the powdery mildew fungus (Hok et al., 2011; Hok et al., 2014). The extracellular region of IOS1 is composed of LRRs and a domain, which shares similarities with malectin from animals. Animal malectins bind carbohydrates and participate in monitoring proper folding of glycoproteins in the endoplasmic reticulum (ER). We observed retention of IOS1 in the ER, which appears to be mediated through the malectin-like (ML) domain. Yeast two-hybrid screens with the extracellular IOS1 domain identified an ER-localized protein. I will present and discuss results from this analysis.

Hok et al. (2014). Plant Physiol. 166, 1506-1518. Hok et al. (2011). Plant Cell Environ. 34, 1944-1957

P-12

Impact of the FXS-linked R138Q mutation on FMRP sumoylation and function

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Fragile X syndrome (FXS) is the most frequent inherited cause of Intellectual Disability (ID) and the best-studied monogenic cause of autism. FXS results from mutations within the FMR1 gene causing the functional absence of the Fragile X Mental Retardation Protein (FMRP). FMRP is an RNA-binding protein that transports mRNAs in RNA granules along axons and dendrites to the base of active synapses for local translation upon neuronal activation. In FXS, FMRP loss-of-function leads to a pathological hyper-abundance of long thin immature

dendritic protrusions. Such defects result from abnormal postsynaptic maturation and pruning, leading to synaptic communication deficits.

Interestingly, we recently demonstrated that FMRP is a sumoylation substrate in the brain (Khayachi et al 2018, Nat Commun). Sumoylation is an essential post-translational modification that consists in the conjugation of the Small Ubiquitin-like Modifier (SUMO) protein to specific lysine residues of target proteins. Sumoylation regulates a wide range of cellular functions, including spinogenesis and postsynaptic differentiation. We showed that FMRP sumoylation is promoted by the activation of metabotropic glutamate receptors and controls FMRP homomerization within dendritic RNA granules prior to regulating spine elimination and maturation.

Noteworthy, some missense mutations in the FMR1 gene have been described in FXS patients, leading to amino-acid changes close to the SUMO sites of FMRP. In the lab, we are particularly interested in the R138Q mutation. In Fmr1-/y neurons, we observed that the re-introduction of the pathogenic GFP-FMRP-R138Q mutant failed to rescue the spine density compared to the WT. This finding opens up the exciting possibility that the FXS-linked R138Q mutation may directly alter the activity-dependent sumoylation of FMRP and its synaptic function. We are currently working on our recently engineered FMRP-R138Q Knock-In (KI) mouse model, which represents a unique opportunity to investigate the molecular mechanisms underlying FXS in patients carrying the R138Q mutation.

Khayachi A et al. Sumoylation regulates FMRP-mediated dendritic spine elimination and post-synaptic maturation (Nat Commun 2018).

P-13

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, demonstrate their tumorogenicity in vivo and decipher the molecular pathways that transduce these invasive properties.

P-15

Novel Insights Into Neuronal RNP Granules Upon Aging

Kavya Vinayan PUSHPALATHA and Florence BESSE

iBV

Tight spatio-temporal regulation of gene expression is achieved at least partly by packaging the nascent transcripts with regulatory proteins into ribonucleoprotein (RNP) granules. In neurons, neuronal RNP granules

transport mRNAs to axons or dendrites, and control their local translation in response to external stimuli. While the post-transcriptional regulatory functions of these supramolecular assemblies have been extensively studied, how neuronal RNP granule assembly and dynamics are regulated in vivo in response to developmental signals or aging remains elusive to date. Yet, proper regulation of their dynamics and size is necessary, as the formation of toxic RNP aggregates has been proposed to disrupt cell ribostasis, leading to neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS).

In the laboratory, we study in Drosophila neuronal RNP granules that are characterized by the presence of IMP, the Drosophila ortholog of ZBP1. These granules localize in the cell body of CNS neurons and are transported selectively to the axons of Mushroom Body (MB) gamma neurons. To study the impact of aging on neuronal RNP granules, we have analyzed IMP granules in flies of increasing age. Strikingly, the IMP granules became more distinct from the cytoplasmic pool with increasing age of analyzed flies. Such an increase did not result from variations in Imp expression levels. Furthermore, the large IMP granules observed in aged flies contained profilin mRNA, and did not colocalize with Ubiquitin or aggregation markers such as p62, suggesting that they do not correspond to protein aggregates. Age-dependent change of Imp granules is also not induced by neuronal activity, as it persisted after inactivation of MB gamma neurons. Finally, no similar change in granule properties was observed for FMRP, Syncrip, PABP granules in aged MB neurons, suggesting a certain degree of specificity. To unravel the molecular mechanisms regulating IMP granule assembly and dynamics during aging, we have started testing the role of cellular pathways altered in aging, including ROS signaling, autophagy, proteasomal pathway and post-translational modifications. Preliminary results suggesting that IMP granules respond to the MAPK phosphorylation pathway, and physically interact with the Drosophila MAP Rolled will be presented.

P-16

The hepatokine FNDC5/Irisin is a protective factor against Non-Alcoholic Fatty Liver

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Fibronectin type III domain containing 5 (FNDC5) is a trans-membrane protein. The proteolytic cleavage of its ectodomain from cell surfaces generates soluble irisin. Initially described as being mainly produced in muscle during physical exercise, irisin mediates brown fat like development and thermogenesis of adipose tissue and regulates carbohydrate and lipid metabolism. The aim of this study was to evaluate the hepatic expression of FNDC5/irisin and its role in hepatocytes in non-alcoholic fatty liver disease (NAFLD). We here reported that hepatic expression of FNDC5 increased with hepatic steatosis and liver injury in mouse models of NAFLD (HFD and MCDD). Hepatocyte FNDC5 expression was mainly dependent on the anti-oxidative pathway p62/NRF2 leading to the secretion of soluble irisin. The down-regulation of FNDC5 in HepG2 increased the expression of key enzymes of fatty acid uptake (CD36), gluconeogenesis (PEPCK), lipogenesis (FAS), esterification of fatty acids (DGAT, SCD) and VLDL synthesis (CIDEB and APOB) leading to increased steatosis and insulin resistance in response to oleic acid and N-acetyl glucosamine, respectively. The downregulation of FNDC5 also sensitized primary hepatocytes to cell death and apoptosis mediated by TNF?. This has been associated with decreased autophagic flux. We then quantified the hepatic gene expression of FNDC5 in human. The hepatic expression of FNDC5 increased with NAFLD and strongly correlated with hepatic steatosis and liver injury in obese patients (n=36). Interestingly, the carriage of FNDC5 rs3480 minor allele was significantly protective for the presence of a significant steatosis (S2-3 versus S0-1) independently of the PNPLA3 rs738409 genotype, age, female gender, BMI and the presence of a type 2 Diabetes Mellitus in a cohort of 613 obese patients. In conclusion, our human

and experimental data strongly suggest that hepatic expression of FNDC5 could dampen the development of NAFLD by negatively regulating the steatogenesis and hepatocyte death.

P-17

Protective mechanisms of anaesthetic preconditionning with halogeneted agents 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 morbidity and mortality. The protective effect against IR by anesthetic preconditioning (APC) by volatile anesthetic agents, in particular sevoflurane, has been widely demonstrated in animal and human models. APC appears to protect myocardial cells from cell death, especially from apoptosis, but the mechanisms involved remain to be identified. Thus, we hypothesized that the anti-apoptotic members of the Bcl-2 family of proteins are modulated by sevoflurane and participate in the cardioprotective effect of APC.

In order to study the mechanisms of APC against myocardial IR, we used a validated in vitro model reproducing IR injury: 2h30 of ischemia followed by reperfusion with a normoxic medium Krebs-Henseleit for 90 minutes.

APC was performed by adding sevoflurane from the stock solution (BaxterÂ®) directly into the culture medium at an initial concentration of 20 mM prior to ischemia for 90 minutes. Apoptosis was measured by caspase activity assay and western blotting by looking at the expression of cleaved caspase 3 under IR and APC conditions.

We have developed an in vitro model to study IR and APC in rat cardiomyoblast (H9c2 cells). Our model faithfully reproduces the protective effect of APC which results a significant decrease in apoptosis in IR condition. We have shown that sevoflurane induces overexpression of the anti-apoptotic protein Bcl-xL in a dose and time-dependent manner, responsible for the protective effect of APC in our cell model with respect to IR injury. We validated this result in vivo from mouse hearts lysates. The rapid increase of Bcl-xL expression induced by sevoflurane in our cell model seems to be posttranscriptionally regulated. Indeed, we showed that sevoflurane, apart from any stress, is able to induce the phosphorylation of Bcl-xL on its serine / threonine residues. On the other hand, we observed that the activation of serine threonine kinase Akt was induced by sevoflurane. Currently we are analyzing its implication in the phosphorylation of Bcl-xL and in the protective effect of APC.

Our study shows for the first time that the protective effect of APC by sevoflurane is mediated by the increased expression of the anti-apoptotic protein Bcl-xL in a potential Akt-dependent manner. This opens new therapeutic perspectives in perioperative management, particularly in cardiac surgery and non-cardiac surgery in patients with high cardiovascular risk.

P-18

Cross-talk between aphid facultative symbiosis and plant nitrogen fixation symbiosis in the Acyrthosiphon pisum - Medicago truncatula interaction

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The aphid is a major agricultural pest because of their asexual unparalleled reproductive capacity and their ability to overcome host defense response and manipulate host plant physiology. The different research showed

that aphid population growth and its impact on plant fitness are strongly influenced by interaction with other partners, such as endophytes and aphid endosymbionts. For legumes, symbiotic-nitrogen fixation (SNF) is most important biochemical processes, which is conducted by a variety of bacteria, in symbiotic association with plants. In this context, it is very important to know whether and how the presence of different facultative symbionts in the pea aphid and the SNF modulate the legume-aphid interaction and vice-versa. A study integrating elements of the ecology, behavior, and gene expression of the different partners has not yet been well-explored. Therefore, cross talk between different interacting species and their respective symbionts adds a level of complexity that remains to be considered. Based on the observed phenotypes (aphid and plant growth, SNF efficiency), my work will define the importance of the facultative symbiosis of aphids and the nitrogen-fixing symbiosis of legumes in the interaction between pea aphid Acyrthosiphon pisum and leguminous plant Medicago truncatula. Based on the observed phenotypes, we will then decipher the mechanisms (defense, metabolism) explaining the influence of nitrogen fixation on aphid traits (growth, metabolism) and associated symbionts (population dynamics, metabolic pathways) and of each aphid facultative symbiont on the NFS efficacy (plant growth, primary metabolism, gene expression).

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P-19

Targeted next generation sequencing with an extended gene panel does not impact variant detection in mitochondrial diseases

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Since the advent of next generation sequencing (NGS), several studies have tried to evaluate the relevance of targeted gene panel sequencing and whole exome sequencing for molecular diagnosis of mitochondrial diseases. The comparison between these different strategies is extremely difficult. A recent study analysed a cohort of patients affected by a mitochondrial disease using a NGS approach based on a targeted gene panel including 132 genes. This strategy led to identify the causative mutations in 15.2% of cases. The number of novel genes responsible for respiratory chain deficiency increases very rapidly.

In order to determine the impact of larger panels used as a first screening strategy on molecular diagnosis success, we analysed a cohort of 80 patients affected by a mitochondrial disease with a first mitochondrial DNA (mtDNA) NGS screening and secondarily a targeted mitochondrial panel of 281 nuclear genes.

Pathogenic mtDNA abnormalities were identified in 4.1% (1/24) of children and 25% (14/56) of adult patients. The remaining 65 patients were analysed with our targeted mitochondrial panel and this approach enabled us to achieve an identification rate of 21.7% (5/23) in children versus 7.1% (3/42) in adults.

Our results confirm that larger gene panels do not improve diagnostic yield of mitochondrial diseases due to (i) their very high genetic heterogeneity, (ii) the ongoing discovery of novel genes and (iii) mutations in genes apparently not related to mitochondrial function that lead to secondary respiratory chain deficiency.

P-20

Telomere Dynamics among Pacific Corals

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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, the rate of this replicative shortening can be modulated by environmental stress. Telomerase is able to balance this loss by lengthening chromosomal DNA's ends. Moreover, telomeres are capped by shelterin, a protective protein complex that prevents unwanted end-to-end fusion, and the activation of the DNA damage checkpoint. Nevertheless, excessive telomeres shortenning will result in unprotected DNA's ends thus leading to senescence and/or apoptosis. Corals are long-lived fixed colonial invertebrates that display symbiosis with photosynthetic micro-algae. Furthermore, corals are hosts of a still largely unknown world of associated bacteria, viruses, and other protists, forming a complex symbiocosm that biologists refer to as 'holobiont'. In a context of global warming coral reefs undergo massive bleaching events, results of a symbiosis break. Whether telomeres dynamic plays a role in the extreme longevity of coral and in their sensitivity to environmental changes emerges as a key question. For 13 years TARA expeditions have sailed across the global Ocean to sample and explore the diversity of marine life. The TARA-PACIFIC expedition aims to describe the microbial diversity of some reef-building corals, in distinct archipelagos across the Pacific to assess how the composition of coral microbial communities contributes to the resilience and vulnerability of 3 coral species, to stress or disease. In order to explore the role of telomeres in the corals' stress response, we will relate stress markers and telomere variation to the holobiont's microbial diversity and to environmental parameters at an uncommon ecological scale.

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P-21

Genome instability initiate introgressions in budding yeast

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Archaic interspecies admixtures are widespread in animals, plants and fungi. These interbreeding events leave genomic segments with distinct ancestry that represent potential source of adaptation to new environments. Introgressions are thought to arise from hybridization process followed by meiotic recombination and subsequent backcrossing. However, this model is difficult to reconcile owning the low recombination and

gametes viability of interspecies hybrid and therefore the molecular mechanisms of foreign DNA integration have remained elusive. Here, we show that genome instability that followed an ancestral interspecies yeast hybridization event is a key mechanism to generate genomic introgressions. We discovered a natural ?S. cerevisiae and ?S. paradoxus hybrid that can be regarded as a “living fossil”, which represent the ancestral state of an extant ?S. cerevisiae ?clade ?that harbour heavy ?S. paradoxus introgressions. This hybrid is characterised by complete haploid subgenomes for each species that remained largely phased but underwent to catastrophic genomic instability. We detected approximately one hundred recombination events, with the vast majority that manifest in the form of sequence identity blocks derived by loss-of-heterozygosity (LOH). We experimentally demonstrated that naturally occurring LOH blocks generate homology regions that can efficiently restore meiotic crossovers and partially rescue gametes viability. Our study revealed that two distinct stages of the hybridization process can surprisingly coexist and underscore the importance of genome instability in mediating interspecies gene flow.

P-22

Rescue of a novel familial hemiplegic migraine folding defective NaV1.1 mutant leads to gain of function: common mechanism?

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The gene SCN1A encoding the Nav1.1 voltage gated sodium channel is the target of mutations responsible for a wide range of epilepsy syndromes and for familial hemiplegic migraine (FHM-3), a rare subtype of migraine with aura. Nav1.1 channels are mainly expressed in GABAergic interneurons, and numerous studies showed that epileptogenic NaV1.1 mutations cause a loss of function of the channel, decreased excitability of GABAergic neurons and reduced inhibition. Differently, we found that mutations responsible for FHM-3 can induce a gain of function of the channel and hyperexcitability of GABAergic neurons. This effect may induce cortical spreading depression, a proposed pathological mechanism of migraine. Interestingly, some FHM-3 NaV1.1 mutants are folding defective and show nearly complete loss of function that can, however, be reverted to gain of function upon rescue of folding defects (Cestèle et al.2013 PNAS 110(43):17546). We have undertaken the detailed functional study of the newly identified L1670W missense mutation causing FHM-3, observing a nearly complete loss of function when studied in a human cell line. Incubation of the cells at 30?C partially rescued the function of the mutant, consistently with folding defects. The partially rescued mutant shows modifications in gating properties that are similar to those observed for the previously studied folding defective FHM-3 mutant. Then, we studied the mutation in primary cultures of neocortical neurons to near physiological conditions. Interestingly, the mutant is constitutively partially rescued in neurons and we showed that the overall effect of the mutation is a gain of function of Nav1.1 channels and hyperexcitability of GABAergic neurons. These results confirm our previously data (Cestèle et al.2013 PNAS) and put forward a possible common mechanism for FHM-3.

P-23

Investigating the role of Ro60/sY-RNAs complex in lipid-laden macrophages and adipocytes

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During obesity progression, lipid-laden macrophages infiltrate in adipose tissue and favor the establishment of a chronic low inflammatory state through the secretion of pro-inflammatory cytokines. Meanwhile, the adipocytes stimulated by these cytokines release saturated fatty acids that induce the production of more pro-inflammatory cytokines by infiltrated macrophages, establishing in this way a crosstalk between these two cellular types that cohexist in adipose tissue. Understanding the crosstalk is of crucial importance, because it participates in the onset of adipose tissue inflammation/disfunction and in the metabolic complications of obesity, such as Insulin Resistance, Type 2 Diabetes and Cardio-metabolic Diseases.

Recently, we showed that in lipid-laden macrophages sY-RNAs, a novel class of small non-coding RNA, associate with Ro60 to act as mediator of inflammatory diseases by regulating the activation of NF-kB and caspase 3-dependent pathways (1, 2); however the molecular mechanisms behind the mode of action of Ro60/sY-RNAs complex are still unknown. Moreover, our preliminary data show that sY-RNAs expression is deregulated in the adipose tissue of obese mice, suggesting that the complex could be a player in macrophages/adipocytes crosstalk, contributing in this way to adipose tissue inflammation and obesity-associated metabolic diseases.

Here we demonstrate that Ro60 changes its intracellular localization in macrophages treated with pro-apoptotic or pro-atherogenic stimuli, translocating to chromatin and binding several class of genomic regions such as repetitive elements (SINEs and LINEs), introns and promoters, indicating that this complex could have an important role in gene expression regulation of inflammatory macrophages. To integrate these results, we will perform RNA-seq experiments in order to understand which pathways are affected by Ro60 regulation during inflammation. Interestingly, we have also found that sY-RNAs levels are modulated during adipocytes differentiation and in adipocytes treated with factors involved in insulin resistance, reinforcing the possibility that these non-coding RNAs could play a functional role not only in inflammatory macrophages, but also in adipocytes pathophysiology. Finally, further experiments will be performed to elucidate if Ro60/sY-RNAs complex could have a functional role in insulin resistance of adipocytes and in macrophages/adipocytes crosstalk during obesity progression.

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P-24

Role of the Circadian Transcription Factor KLF10 in the development of Non Alcoholic Fatty Liver Diseases

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Non alcoholic Fatty Liver diseases (NAFLD), ranging from simple steatosis to steatohepatitis, fibrosis and finally hepatocellular carcinoma, is a widespread hepatic metabolic disorder. The circadian system is a main

coordinator of hepatic metabolism and clock disruption has been associated with the NAFLD development. We previously showed that the hepatic expression of the transcription factor Krüppel like factor 10 (Klf10) gene is directly regulated by the circadian clock and controls circadian expression of genes related to carbohydrate and lipid metabolism. We here investigate the potential contribution of KLF10 in NAFLD development. We report that hepatic KLF10 expression occurs in hepatocytes and the non parenchymal fraction and increases with liver complications in a mouse model of steatohepatitis. (Methionine and Choline Deficient Diet, MCDD). The global deficiency of KLF10 is associated with more severe steatosis and the upregulation of markers of lipogenesis, lipid droplet synthesis and oxidative stress compared to control mice in response to MCDD. These, in turn, are associated with increased liver injury (as evaluated by the transaminase activity) and the priming of fibrogenesis. Hepatocyte KLF10 could mainly drive these effects since hepatocyte-specific Klf10 knockout mice display more liver injury and elevated hepatic expression of markers of oxidative stress, lipid droplet synthesis and fibrosis. Finally, we also show that the hepatic expression of KLF10 increases with NAFLD and strongly correlates with hepatic steatosis (rs=0.565, p<0.001, n=31) and liver injury (ALT, rs=0.437, p=0.013, n=31) in obese patients. We believe that KLF10 could be a protective factor against the development of NAFLD and work is in progress to decipher the mechanisms by which KLF10 acts.

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P-25

GENEXPOSOMICS Linking genetic to environmental factors for prevention of lung cancer

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Population densification and ageing pose a new risk for cancer development: Exposure to concentrated air pollution. Previous studies have shown that air pollution is strongly associated with mortality from lung cancer. In France, the prevalence of chronic inflammation in the respiratory tract (COPD and emphysema) and lung cancers has reached epidemic proportions with over 3 million adults being affected. The rates of COPD and lung cancers are aggravated in the elderly. Although critical, there are little, if any, therapeutic options to alter disease progression from inflammatory COPD to cancer. As life expectancy increases and the baby-boom generation reaches retirement age, the already heavy burden that these two endstage lung diseases impose on the health care system (â‚¬50 billion/year) is set to increase dramatically. Air pollution and particularly the particulate matter are known carcinogens. However, the causal pollutant components and physiologic mechanisms underlying inflammation and carcinogenesis are not understood.

To produce high-quality scientific research data "GENEXPOSOMICS" will regroup a multidisciplinary network of well-known specialists which altogether will: (1) Use biobanks to provide a deep characterization of autophagy related gene sequence variations of 1000 nonsmoker patients, living in 17 French cities with varying levels of traffic and industrialised pollution; (2) Define Environmental and Genetic Risk factors of lung diseases as a first step towards personalised medicine for the benefit of French citizens and future generations; (3) Develop new models and methodologies for predictive and mechanistic environmental carcinogenicity; and (4) Propose paths for diagnostics, prevention and therapeutic approaches.

This will improve cognition of the risk of lung diseases that is promoted by air pollution and help to solve the conflict that can exist between economic development and human health preservation.

P-26

Otx2: a neuroprotective signal for Adult Retina Photoreceptors?

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Photoreceptors (PRs) are specialized retinal neurons responsible of the phototransduction, the conversion of the light in electrical stimuli sent then to the brain. Their dysfunction and/or death are the cause of different retinal diseases, affecting seriously the quality of the life of the patients. Then, the research of neuroprotective mechanisms underlying PRs viability and functionality can be a valid strategy to develop medical therapies.

PRs feeding and nursing depends on the adjacent melanin-containing compartment: the Retinal Pigment Epithelium (RPE). Both PRs and RPE express the Otx2 homeoprotein transcription factor, a major actor of eye and retina development from the early embryonic stages (RPE specification, PRs identity, bipolar differentiation) (Béby et al., 2013; Nishida et al., 2003). Less clear is the role of this protein after retina development is completed.

Conditional depletion of Otx2 in adult retina leads to RPE dystrophy and PRs degeneration. Surprisingly, RPE-specific Otx2 ablation leads to PRs degeneration and, in a condition of full retina Otx2 KO, the re-expression of the an exogenous Otx2 in the RPE rescues PRs viability. These data suggest a non-autonomous RPE-based neuroprotective mechanism, where the role of Otx2 has to be elucidated (BÃ©by et al., 2010; Housset et al., 2013). Several studies showed the capacity of several homeoproteins, including Otx2, to travel between different cells population, acting as signal factors. Then we want to test if RPE can be an exogenous-Otx2 source for the PRs and if the transferred protein can have a neuroprotective effect. To test whether the RPE-expressed Otx2 could really transfer to PRs, we will introduce a tagged-Otx2 protein in RPE cells in vivo and look if we can detect it later in PRs. In parallel we will investigate the intrinsic function of Otx2 in PRs using a mouse driver line to specifically disrupt the Otx2 gene in PRs. This will help us to better understand the role of RPE-derived Otx2 on PRs survival and on PRs gene expression in adult mouse retina.

P-27

RSPO1: a key player for proper endocrine cell function 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, ?-, ?-, ?-, PP- and ?- 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 ?-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 1,2. Having identified members of cWNT in a screen seeking for inducers of pancreatic beta-cell neogenesis, we focused on RSPO1, a secreted protein and one of the main players of the cWNT molecular network. We thus analyzed Rspo1 full knock-out mice (Rspo1-/-). Combining immunohistochemistry, RNA scope, RT-qPCR and ELISA approaches with functional studies, we show that Rspo1 is exclusively expressed in the acinar compartment and provide evidences suggesting a paracrine role of this protein in regulating pancreatic hormones production and secretion. Importantly, we also show that the Rspo1 loss-of-function results in an improved glycemic control upon glucose challenge, despite a significant reduction in insulin secretion both at basal level and upon glucose injection. Together, our results suggest that RSPO1 is a key paracrine factor for proper endocrine cell function and that strategies aiming at controlling its expression could be beneficial for diabetes research.

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P-28

VDAC1 and ciliopathy

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The Voltage-dependent anion channel 1 (VDAC1) is the major mitochondrial channel protein of the outer membrane that controls both cellular metabolism and apoptosis. Any modifications of VDAC1 activity and/or structure can influence the fate of the cells and, in particular, of cancer cells (1).

We previously found a new post-translational modification of VDAC1, which was linked to a higher metabolic capacity of cancer cells exposed to a long-term hypoxia, a feature of locally advanced solid tumors. The molecular mechanism of VDAC1 modification is hypoxia-inducible factor (HIF) and TP53/TP73-dependent and involves direct contact between mitochondria and endolysosomes. This crosstalk between organelles leads to truncation of VDAC1 (VDAC1-?C) by lysosomal peptidases and to increase resistance to chemotherapy (2,3).

We now found a novel mechanism linking VDAC1-?C to the inhibition of primary cilia biogenesis in MEF-RAS VDAC1-/- cells (Mouse Embryonic Fibroblast cell model) in which we restore i) wild type VDAC1 or (ii) VDAC1 mutated at the VDAC1-?C cleavage site. The primary cilium is an immotile organelle of most cell types that senses the extracellular environment to regulate intracellular signaling of multiple cell processes. Defects in the structure and function of primary cilia lead to a range of multifaceted disease phenotypes termed ciliopathies. Since primary cilia have the ability to influence cell cycle and modulate cilia-related signaling transduction, dysfunction or disruption of cilia has long been proposed as a prerequisite step of cancer development. In this view, cancer is included among ciliopathies (4,5).

The most common form of kidney cancer, the clear cell renal cell carcinoma (ccRCC), is characterized as a ciliopathy with loss of the primary cilium (6), in both in vitro and in vivo in ccRCC cohorts of patients. Interestingly, we found the same correlation between the presence of VDAC1-?C and the inhibition of ciliogenesis in the ccRCC. Finally, we established a hypoxic gene expression signature to predict the presence of VDAC1-?C and the absence of primary cilia that can be a prognostic and predictive marker in ccRCC.

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P-29

The impact of various N- and C-truncated A? peptides in Alzheimer's disease.

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Alzheimer's disease (AD) is the most common form of age-related dementia in the elderly in the world. Recent advances allowed a better understanding of the putative causes of disease onset and progression. It is well known that proteinaceous aggregates invade AD-affected brains and, at least, could contribute to the overall degenerative process leading to cognitive impairments and more particularly, memory loss. These lesions are essentially composed of insoluble peptides referred to as amyloid ?-peptides (A?). Numerous studies aimed at understanding why A? peptides, that are physiologically occurring fragments in normal brains, accumulate in AD brains. This aroused the interest in delineating the mechanisms by which A? peptides are generated and main enzymes involved in this process have been identified. It is expected to interfere with disease progression by blocking A?-generating or A?-degrading enzymes by chemical design of blockers or activators, respectively. More recently, this simplistic view of ?APP processing has been complicated by the demonstration that A? peptides could themselves undergo secondary cleavages yielding fragments that appear sometimes even more toxic that their parent counterpart and thus, their pathological "weight" could well have been under-estimated. The project aimed at studying the nature of enzymes involved in these secondary cleavages and their influence on the pathology. We propose to examine the implication of several enzymes (Aminopeptidases A, M et dipeptidylpeptidase IV) in the cleavage of A? peptides and the potential beneficial influence of their blockade in cells. Our project will be extended to in vivo models of transgenic mice harboring most of the AD-related biochemical, anatomical and cognitive stigmata. Hopefully, our project should lead to the delineation of novel therapeutic tracks and to the design of new therapeutic probes able to interfere with the AD-associated neurodegenerative process.

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P-30

Control of brite adipogenesis by fatty acid metabolites

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The increasing prevalence of overweight and obesity has reached "epidemic" proportions with 2 billion of overweight persons (BMI>25 kg/m²) and 650 million of obese persons (BMI>30 kg/m²) in the World (World Health Organization). In most cases, this situation is induced by imbalance between entries and energy

expenditure leading to increase of white adipose tissue (WAT) mass. The recent discovery of functional brown and brite (brown-in-white), thermogenic adipocytes, in adult humans has led to the consideration of their use to increase energy expenditure in the treatment of overweight, obesity and associated diseases. Lipids and fatty acids stored into adipocytes are the main substrates for adaptive thermogenesis but are also involved in adipose tissue development and function. Differences in fatty acid composition of dietary fat and relative intake of ?6 to ?3 poly-unsaturated fatty acids (PUFAs) contribute to adipose tissue development. Quality and quantity of dietary PUFAs control the kind of metabolites synthetized and these are involved in several physiological and inflammatory processes. We previously showed that arachidonic acid (ARA), a ?6 PUFA, and some of its metabolites named oxylipins, are able to inhibit or induce britening. We aim to detect distinct oxylipins in human cell models and in murine and human adipose tissue biopsies, which are associated with brite adipogenesis and thus potentially able to increase energy expenditure. We identified 2 interesting metabolites, derived from ?6 PUFA linoleic acid: 9- and 13-Hydroxyoctadecadienoic acid (HODE). Using our human cell model (hMADS cells), we showed that these compounds did not induce any direct britening of white adipocytes. However, the, inhibition of their synthesis pathway induced a decrease in adipogenic and key brown adipocyte gene expression which was fully reversed when the medium was supplemented with 9- and 13-HODE. These observations are in favor of 9- and 13-HODE playing directly or indirectly an important role in the control of brown adipogenesis. Further studies are required to decipher the involved mechanisms.

P-31

Functional characterization of long non-coding RNAs modulated by hypoxia in Non-Small Cell Lung Carcinomas

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Lung cancer is the leading cause of cancer death worldwide, with poor prognosis and a high rate of recurrence despite early surgical removal. It is therefore essential to identify new prognostic markers and new therapeutic targets. We are interested in gene regulation related to hypoxia, a factor associated with relapse of lung adenocarcinomas (LUAD). While long non-coding RNAs (lncRNA) are increasingly recognized as major gene expression regulators through various molecular mechanisms, their roles in cancer development and hypoxic response are still largely unexplored. Combining profiling studies on A549 LUAD cell line cultured in normoxic or hypoxic conditions and on early stages LUAD biopsies, we identified a signature of lncRNAs that are regulated by hypoxia in vitro and correlated to i) the hypoxic status of tumors and/or ii) the overall survival of patients. We focused on two candidates, NLUCAT1 and LINC01116, and confirmed their dysregulation on public datasets from larger LUAD and LUSC (Lung Squamous Cell Carcinomas) cohorts of "The Cancer Genome Atlas". We showed that NLUCAT1 is a large nuclear transcript composed of 6 exons whose CRISPR/Cas9-mediated deletion in LUAD cells revealed a decrease in proliferative and invasive properties, an increase in oxidative stress and a higher sensitivity to cisplatin-induced apoptosis. Through transcriptome analysis, we identified 4 genes of the NRF2-regulated gene network that were downregulated in NLUCAT1 knockout cells and we demonstrated that their concomitant RNA interference partially mimicked the consequences of NLUCAT1 inactivation on cisplatin-induced apoptosis. On another hand, LINC01116 is a short cytosolic transcript composed of 3 exons. Using RNA FISH imaging, RNA interference knock-down and transcriptome analyses, we suggest its potential implication in cellular morphology and cell-to-cell contact. In addition, we found a correlation between LINC01116 expression and an alpha catenin gene network involved in cell-to-cell signaling.

P-32

Btk toxins influence progenitor cell fate of intestinal stem cell

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In order to reduce the use of chemical pesticides, organic farming are using biopesticides.

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 in these crystals are called Cry endotoxins and have been known for decades to display insecticidal activity against specific insect groups by destroying their gut and leading to their death by septicemia.

My PhD project aims to study the impacts of Btk on the gut homeostasis of non-target organisms. For that, I will use the powerful laboratory model Drosophila melanogaster.

In the Drosophila midgut, Intestinal Stem Cells (ISCs) are required for maintenance of the proper cell composition in the adult intestine. To ensure permanent recruitment of newly differentiated cells, ISCs undergoe 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. Upon a bacterial intoxication that causes gut damages, intestinal cell renewing is accelerated and most of progenitor cells differentiate into enterocytes to replenish the damaged ones.

Strikingly, our work revealed that the number of enteroendocrine cells (EEC) increases after an intoxication by the commercialized form of Btk despite the damages caused to the enterocytes. We have shown that this EEC increase is dependent on the Cry toxins.

My work is to understand how Btk induces an increase in EECs at the expense of enterocyte differentiation and the putative link between Cry toxins and the inhibition of Notch pathway.

Key-words: Bioinsecticides, Bacillus thuringiensis (Bt), Drosophila melanogaster, intestinal homeostasis , Cry toxins , Notch pathway .

P-33

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

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Melanoma cells are known for their plasticity and ability to phenotype switch toward an invasive de-differentiated mesenchymal state leading to an aggressive clinical behavior.

The emergence of this mesenchymal phenotype has been shown during primary, adaptive and acquired resistance to MAPK-targeted therapies in BRAF mutant melanoma cells.

The mesenchymal acquired resistance is achieved by overexpression of receptor tyrosine kinases and we recently 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 poorly understood phenotype remains to be defined. In this perspective, our study focused on the analysis and characterization of a pool of microRNAs (miRNAs) involved in fibrotic diseases, acting as pro-fibrotic or anti-fibrotic regulators, called "FibromiRs"?.

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 potential contribution in acquisition of invasiveness.

Moreover, we analyzed its biological role and regulation in response to BRAF and MEK inhibitors treatment. The two miRNAs are found strongly overexpressed in mesenchymal resistant cells versus sensitive cells, and induced upon TGF-? 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-? 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.

Ectopic expression of either miRNA triggers activation of the ECM signature and induction of mesenchymal traits related to activation of STAT3, whereas inhibition of miR-143 or miR-145 impairs the up-regulation of ECM components and remodeling proteins induced by MAPK pathway inhibition. Overall, our data indicate that the miR-143~145 cluster contributes to phenotypic 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.

Nazarian et al., 2010

P-34

Organelle Dynamics During Fungal Filamentous Growth

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Candida albicans is a major opportunistic human fungal pathogen that causes superficial and systemic infections. The capacity of this organism to switch from budding to filamentous growth is critical for its ability to invade and colonize different environments, such as human tissues, and hence for its pathogenicity. The localization of several components of the hyphal tip growth apparatus have been described [1]. Despite this, there is a lack of understanding how these components are organized with respect to one another and how this organization changes during filamentous growth. Specifically, are the dynamics of different membrane compartments coordinated to facilitate sustained polarized filamentous growth? To address this question, we have optimized four spectrally distinct fluorescent proteins for live-cell imaging in C. albicans, which makes it now feasible to simultaneously visualize multiple compartments during filamentous growth. As a result, we have been investigating how membrane trafficking and tip elongation components are organized and coordinated during hyphal growth. A central question is whether this organization is altered upon invasive filamentous growth. To address this, we are using an invasion model composed of polydimethylsiloxane (PDMS) micro-chambers [2], in which invasive filamentous growth can be easily followed from entrapped C. albicans cells. The stiffness of this polymer can be varied, resulting in different resistive forces for C. albicans cells [3]. I have begun to examine organelle dynamics in hyphal filaments growing invasively and intend to investigate this process with different PDMS resistive forces, in order to mimic the physical forces that C. albicans encounters during host invasion. We will also examine the relationships between organelle dynamics and cell shape during invasive growth in PDMS

micro-chambers of varying stiffness. These studies should provide a global understanding of polarized growth in the context of invasion by a major human fungal pathogen.

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P-35

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

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Liposarcomas (LPS) are aggressive adipose tissue tumors mainly represented by well-differentiated (WDLPS) and dedifferentiated (DDLPS) LPS subtypes. Standard chemotherapy and current 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 JNJ-4275693. For this study, our team collaborates with 3 other teams, experts in surgical pathology, biostatistics and oncology.

Expression studies

Using immunohistochemistry, we have already analysed the expression of FGFR1-4 in 201 liposarcoma primary tumors (74 WDLPS and 127 DDLPS). We showed that FGFR1 and FGFR4 are the main expressed FGFRs, which is consistent with the expression levels we have detected in our panel of 5 cell lines (2 DDLPS and 3 WDLPS) by immunohistochemistry and western blotting.

We will perform a correlation study between the FGFRs expression and clinical data (overall survival and progression-free survival).

Functional studies

We studied the sensitivity of these 5 cell lines to JNJ-4275693 and demonstrated a significant inhibitory effect on viability, induction of apoptosis and cell-cycle arrest. JNJ-4275693 treatment significantly inhibited the phosphorylation of p42/p44 (MAPKinase pathway) whereas the effect on the phosphorylation of AKT (PI3K/AKT/mTOR pathway) was less consistent. In order to antagonize the PI3K pathway, we are also testing the effect of the PI3K/mTOR inhibitor BEZ235 alone or in combination with JNJ-4275693.

Our preliminary results are convincing and encourage us to pursue with the following steps: the analysis of i) the effects of modulating FGFR1 and FGFR4 expression on cell proliferation, cell-cycle and apoptosis using an siRNA approach and ii) the mechanisms of sensitivity and resistance to JNJ-4275693 (by phosphoproteomic analysis). Our promising in vitro results will be tested in vivo, using xenograft models.

P-36

With a little help from my friend: a nested LINE-1-Sleeping Beauty system to measure LINE-1 retrotransposition from chromosomal positions

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Facioscapulohumeral Distrophy (FSHD) affects 1 in 8000 individuals worldwide. This rare disease is linked to the expression of DUX4, a homeobox protein only expressed in testis and in the 2 cell-like embryos. When expressed in muscle cells, this protein induces apoptosis, leading to loss of muscle tissue. DUX4 has also been found to bind and activate the transcription of transposable elements. Among these, 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 and in some somatic tissues such as the brain.

In this project, we would like to study the possible link between DUX4 expression and LINE-1 retrotranposition on FSHD affected myoblasts. A popular L1 retrotransposition assay in cultured cells is based on a plasmid-borne retrotransposition-competent L1 carrying a reporter gene, which becomes functional only upon splicing, reverse transcription and integration. However, variations between transfection efficiencies and plasmid copy number render this system difficult to use for comparing retrotransposition levels among different cell types.

To avoid these limitations, we developed a novel retrotransposition assay based on a Sleeping Beauty DNA transposon vector. In this assay, the reporter L1 copy is randomly integrated in the genome and L1 retrotransposition can be induced by adding doxycyclin to the culture medium. Here, we show L1 mobility on murine myoblasts and human primary myoblasts. In the future, we expect to use this system to study L1 retrotransposition through human primary myoblast differentiation.

P-37

Modulation of Lysosomal-Autophagic Pathway in Alzheimer's Disease

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Alzheimer's disease (AD) belongs to the family of proteinopathies that are characterized by the accumulation of specific aggregated proteins in the brain. The Lysosomal-Autophagic (L-A) pathway is known to play a major role in the clearance of aggregated proteins and extensive research has therefore focused on enhancing this degradation pathway. AD has been mainly associated with the aggregation of extracellular Aß and intracellular Tau. However, recent work showed in a mouse model of AD (3xTg), an early accumulation of intra-cytoplasmic aggregates, corresponding to C99, the precursor of Aß. These aggregates were found to accumulate within autophagic vesicles and to be the consequence of a defective L-A function. The aim of my PhD work is to investigate the role of the transcription factor EB (TFEB), a major enhancer of L-A biogenesis, in preventing both early C99 accumulation and AD-related phenotypes. We decided to use a genetic approach, in which TFEB is overexpressed by viral expression. Before in vivo experiments, my first aim was to validate the effects of TFEB not only on its target genes but also on C99 accumulation in SH-SY5Y-APPswe and HEK-APP/BACE cells, both known to accumulate C99. Indeed, these in vitro data confirmed the earlier described effects of TFEB on L-A markers, but also revealed a clear TFEB-mediated reduction of C99 accumulation. Accordingly, TFEB overexpression led to a particularly strong increase in the expression of cathepsins, the main C99 degrading enzymes. Then, to investigate the effect of TFEB in vivo, AAV8-CMV-TFEB virus was introduced by unilateral intra-cerebro-ventricular in newborn mice and C99 expression was analyzed at later stages at which it should be

easily detected. These data validated an expression of transcriptional active TFEB in many brain regions at least until 9 months, but unfortunately a too low TFEB expression was seen within C99-accumulating brain areas (subiculum) to analyze its impact on C99. To more specifically target TFEB expression, some mice were stereotaxically injected in the subiculum at 3 months of age. In these animals, we confirmed a drastic reduction in C99 levels within TFEB expressing neurons validating TFEB as a promising strategy to enhance the degradation of C99 aggregates in AD model. My future studies aim to confirm the effects of TFEB on the accumulation of C99, but also of other aggregated proteins implicated in AD and its impact on AD-related behavioral phenotypes.

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P-38

Unravelling the physiopathological actions of the newly discovered A? peptides in the brain

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The APP (amyloid precursor protein), an integral membrane protein, has two known splicing pathways: a non-amyloidogenic and amyloidogenic one. The latter received a lot of attention in recent years due to the production of A? (amyloid ? protein) and its association with Alzheimer's disease. As A? was thought to be the only neurological active derivate, clinical trials for Alzheimer's disease aimed to block the amyloidogenic pathway to stop the process of dementia without success. My work focuses on A? (amyloid ? protein), a newly discovered protein derived from APP processing first described in "?-Secretase processing of APP inhibits neuronal activity in the hippocampus" (Willem et al., 2015). Here, they describe that the long form of A? named A?-? alters neurological activity in the hippocampal CA1 region ex vivo and in vivo. This is intriguing as processing of APP occurs in a competitive manner thus blockage of the A? pathway increases A? production. Therefore, we are interested in A?'s role in cognition and if it leads to deficits in memory formation. To answer this question, we proposed two aims: does chronic A? over-expression or do acute injections of sA?-? (synthetic A?-?) into the hippocampal CA1 region lead to memory deficits. For the first aim we used a transgenic mouse line "MISEPA2" to test for memory deficits conducting a series of behavioural tests (Morris Water maze, Contextual Fear Conditioning and Actimeter). For testing the acute effects of sA?-?, 6-weeks old C57BL/6 mice underwent stereotaxic surgery to implant cannula guides into the hippocampal CA1 region. After a recovery period sA?-? was injected directly before Contextual Fear Conditioning testing. My poster will give an overview of my results obtained so far.

Secretase processing of APP inhibits neuronal activity in the hippocampus. Nature, 526(7573), 443–447. https://doi.org/10.1038/nature14864

P-39

Study Tracking cell trajectories by single-cell transcriptome analysis during airway epithelium regeneration

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Key words: airway epithelium, multiciliogenesis, differentiation, progenitor/stem cells, gene expression.

The upper airway epithelium is mainly composed of 3 cell types: multiciliated (MCCs), goblet, and basal cells. After respiratory injuries, the tissue must repair to reproducing reproduce a functional mucociliary epithelium through processes of proliferation and differentiation of a subpopulation of progenitor cells1. In chronic airway diseases, such as chronic obstructive pulmonary disease or asthma, the injured epithelium frequently displays defective repair and remodeling characterized by a loss of MCCs with goblet cell hyperplasia leading to mucus hyper-secretion2. No therapy has proven to be efficient to reduce the remodeling of the tissue mostly because the accurate molecular events governing the differentiation of the epithelium have not yet been characterized.

My project aimsWe are aiming at at characterizing the sequence of cellular and molecular events taking place during airway mucociliary epithelium). Taking the advantage of deep sequencing and sSingle cell transcriptomics make possible the accurate characterization of subpopulations through single cell transcriptome analysis.with high resolution.

We wereAfter having reported, for the first to time, publish single cell transcriptomes analysis of in vitro regenerated differentiated airway epithelium4, were we havewe developed a new robust clustering method, which enabled the identification of new transitionary cell types corresponding to key states in regeneration of the human airway epithelium. ,Our results allow us to track cell trajectories from progenitor cells to differentiated cells, with insight in the key regulatory processes involved in cell fate decision.

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P-40

Preclinical evaluation of Alexidine in Resistant Chronic Myelogenous Leukemia and Relapses after Tyrosine Kinase Inhibitors discontinuation

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Abstract : 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 recently described molecular mechanisms leading to eradication of those LSCs (Mourgues, Leukemia 2015) and by a bioinformatic approach identified a compound, Alexidine (ALX), able to stimulate those mechanisms. Our in vitro (CML cell lines) & ex vivo (primary cells from CML patients) experiments showed strong evidences of ALX in inducing cell death. Indeed, ALX 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 itâ€™s impact on primitive stem/progenitor cells (CD34+38-) from sensible and TKI resistant patients.

Moreover, our last experiments suggest a role of ALX in the respiratory chain we are currently investigating.

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/?c-/-) mice to test the capacity of ALX 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 will be 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 ALX in combination with TKIs.

The BMI1 polycomb protein represses cyclin G2-induced autophagy to support proliferation in chronic myeloid leukemia cells (Mourgues et al, Leukemia 2015)

P-41

The impact of intronic LINE-1 insertions on host gene expression

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Transposable elements (TEs) account for approximately 45% of the human genome.

The only autonomously active TE in humans belongs to the long interspersed element-1 (LINE-1 or L1) family, which comprises 17% of the human genome. More specifically, only the youngest and human-specific subset (L1HS) can replicate in modern humans, while older members of this family are remnants of ancient mobilization events. Of note, L1 replication can impact human health by producing disease-causing germline mutations, or by leading to somatic mosaicism or genomic instability in a variety of cancer types.

Of the 129 reported pathogenic germline L1 insertions in humans, 8 have been located within intronic sequences. However, the number of pathogenic intronic L1 insertions is probably underestimated due to a particular focus on exome sequencing, which exclude these regions. Such intronic insertions can influence cellular gene expression in various ways. Hypothetical or demonstrated mechanisms comprise exon skipping, exonization of intronic sequences, including L1 pieces of sequence, transcription elongation defects, alternative transcription initiation, or long-non-coding RNA generation.

To study the molecular mechanisms by which intronic L1 insertions impact gene expression, we are creating a cultured cell model of a historical pathogenic L1 insertion into intron 1 of the RP2 gene (L1RP), identified in a Retinitis pigmentosa patient, a disease causing gradual vision loss. This is achieved by CRISPR-Cas9-mediated genome engineering in immortalized retinal epithelium cells. Subsequently, we will perform a genome-wide suppressor screen to identify cellular factors contributing to the loss of RP2 expression in this cellular model. We will present our ongoing effort to generate this cellular model.

P-42

Understanding the role of Arf6 in the wingless signaling pathway

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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 ?-catenin destruction complex. This stabilizes cytoplasmic ?-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. Activated Arf6 then mediates the disassembly of the adherens junctions complex leading to a reduction in cell adhesion and an increase in invasiveness and metastasis.

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. Using this model, we hope to find whether the same mechanisms governing the metastasis and invasion of tumours is involved under physiological conditions, and in doing so uncover novel players in this process.

P-43

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 diversity and phenotypic in the offsprings. This process is evolutionary conserved among all eukaryotes, including single cell yeasts. However, sporulation is not always efficient to yield viable segregants, in particular in hybrids, and in industrial yeast strains, due to multiple genetic factors (e.g. divergent genome architecture, gross chromosomal rearrangements, aneuploidy, gene incompatibilities, etc..) hence, limiting the possibility to improve these strains upon classical genetic breeding strategies.

In this work, we take advantage of the natural process called Return to Growth (RTG), in which cells that enter meiosis are able to return to mitotic growth, and yield recombinant products without chromosome 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 variations. The RTG process is induced by a shift in the culture media during the prophase of meiosis and does not generate GMOs. Moreover RTG cells had the same ploidy as the parent, and RTG recombination occurs at multiple sites in the genome leading to extensive loss of heterozygosity (LOH) regions. With this work, we explore how the RTG

framework can be applied in complex artificial and industrial hybrids to generate genetic and phenotypic variability. Moreover, we can use the libraries of recombination RTG hybrids to map quantitative traits loci (QTLs) in RTG cells, opening the possibility to map traits in sterile hybrids not amenable to classical linkage analysis.

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P-44

Deciphering the molecular mechanisms involved in senescence of choroidal melanocytes

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Choroidal melanoma (CM) is the most common primary ocular malignancy in adult population. Half of patients will develop metastases which usually involve the liver. The survival rate at the metastasis stage is only 4 to 5 months. Currently, treatment of primary CM relies mainly on surgery and on radiotherapy. There are no approved systemic treatments for CM once it has spread.

In the majority of cases CM originates from a normal melanocyte but it can also derive from a pre-existing nevus. In the skin, nevus is a lesion that is thought to be formed of melanocytes that entered the process of senescence in response to oncogenes such as BRAFV600E. BRAFV600E represses the expression of MITF, a transcription factor that plays a key role in the melanocyte lineage through controlling the process of proliferation and DNA repair. Our lab showed that MITF suppression promotes senescence of skin melanocytes.

Cellular senescence represents a potent barrier to tumor progression. Nevus can remain static for decade and never progress to malignancy unless other genetic alteration occurs, allowing melanocytes to bypass the process of senescence and restart proliferation. Whether choroidal nevus is also formed of senescent melanocytes remain unknown as well as the mechanisms by which a choroidal nevus might progress to CM.

CM is characterized by oncogenic mutations in the heterotrimeric G protein GNAQ and GNA11. CM also display germline and somatic mutations in BRCA1-.?associated protein 1 (BAP1), which gene maps to chromosome 3, frequently deleted in these tumors. Monosomy of chromosome 3 and BAP1 loss are associated with metastasis and poor prognosis. Interestingly, MITF is also located on the chromosome 3. MITF loss could trigger DNA damage and senescence of choroidal melanocytic cells.

However, reduction of both BAP1 and MITF levels might dampens accurate DNA repair, favoring chromosomal instability and CM progression.

In BAP1 positive, choroidal melanocytic cells, I showed that loss of MITF promotes a senescent phenotype associated with morphological and biochemical changes, a cell cycle arrest and DNA damages. Further I observed that MITF suppression by siRNA induced a decrease in BRCA1 expression, which is known to be involved in the repair of DNA damage.

In conclusion, my results point out to a role of MITF in controlling DNA repair and proliferation in choroidal melanocytes.

P-45

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

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Melanoma is an aggressive skin cancer. It often starts his metastatic journey through lymphatic vessels and first reaches regional lymph nodes (LN). The LN is a crucial crossroad giving access to the blood circulation and the spread of metastatic cells to distant organs [1]. At this late stage, surgical removal is not possible anymore and current therapies fail in half of the patients.

LN is a key tissue of the immune response. Mechanisms rendering the LN permissive to tumor invasion and development are poorly understood but involve complex relationships between tumor cells and the LN microenvironment. Factors secreted by tumors are known to educate the microenvironment of the future metastatic niche before the arrival of metastatic cells [4]. Our team focuses its attention on this first key metastatic step and I am particularly interested in the pre-metastatic education of LN fibroblasts by melanoma-secreted factors.

Fibroblasts Associated with Cancers (CAF) are key components of the tumor microenvironment. They secrete pro-tumorigenic growth factors and remodeled the extracellular matrix. CAF are constitutively activated by factors secreted by tumor cells and acquire contractile properties [2]. In the LN, fibroblasts are called Fibroblastic Reticular Cells (FRC). They are spontaneously contractile, and regulate the LN architecture as well as the recruitment and survival of immune cells [3]. In a mice model of pre-metastatic education of the LN niche, we observed that the injection of factors secreted by melanoma cells induced a drastic enlargement of the draining LN, associated with a decrease of the LN stiffness

Using human primary FRC and melanoma cell lines, my aim is to better understand this phenomenon in vitro. My goals are to identify (A) the effect of factors secreted by melanoma cells on the FRC contractile behavior and (B) the tumoral factors and signaling pathways involved. My results show that factors secreted by melanoma cells with an invasive signature (AXLhigh MITFlow) inhibit FRC contraction. This is associated with major cell morphological changes, the cytoplasmic relocation of the co-transcription co-factor YAP and the inhibition of the JAK/STAT-3/ROCK pathway. YAP plays a key role in mechanotransduction and its nuclear localization is associated with cell contraction. Identifying molecules involved in the inhibition of FRC contraction by melanoma lead to the identification of potential early metastatic biomarkers or therapies.

This work is supported by the Ligue Nationale contre le Cancer, the Canceropôle PACA, the Association Vaincre le Mélanome, and the Fondation ARC. (1) Brown M, Assen FP, Leithner A, Abe J, Schachner H, Asfour G, Bago-Horvath Z, Stein JV, Uhrin P, Sixt M, Kerjaschki D. (2018). Lymph node blood vessels provide exit routes for metastatic tumor cell dissemination in mice. Science 359, 1408-1411. (2) Kalluri, R. (2016). The biology and function of fibroblasts in cancer. Nature Reviews 16, 582-598. (3) Fletcher, A.L., Acton, S.E., and Knoblich, K. (2015). Lymph node fibroblastic reticular cells in health and disease. Nature reviews Immunology 15, 350-361. (4) Liu, Y. et Ciao, X. (2016). Characteristics and Significance of the Pre-Metastatic Niche. Cancer Cell 30, 668-681.

P-46

Parsing the role of LDTg projections in stress-related disorders

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While the stress response is an adaptive mechanism that is beneficial to an organism under a given threshold, it can become maladaptive and expose the organism to harmful outcomes when it is excessively long-lived or intense. Notably, stress is the main factor contributing to stress-related disorders in many species. Defensive behaviors are a subset of behavioral adaptations to environmental stressors, which typically include fight, flight and freezing responses.

We recently identified the laterodorsal tegmental nucleus (LDTg), a hindbrain structure, as a key brain region sensitive to stressful experiences. As such, we found that chronic social stress triggered hyperactivity of cholinergic LDTg neurons. These cellular adaptations led to enduring behavioral changes, including social withdrawal and anhedonia, reminiscent of depressive-like disorders. This clearly positions the LDTg as a key node of the stress response. However, the nature of the stressor is likely to differentially impinge on LDTg neurons. In this PhD project, I aim at exploring the role of the LDTg in processing defensive behaviors combining behavioural and anatomical studies using transgenic mice and viral tools to selectively manipulate LDTg neurons activity.

Using electrical foot shocks to elicit freezing defensive behaviors in mice, we showed that pharmacogenetic inhibition of LDTg neurons during the shocks reduced freezing behavior. Importantly, this was not due to changes in anxiety levels, pain sensitivity or memory of mice. To reveal the identity of the LDTg neurons mediating this effect, we used transgenic mouse lines allowing the expression of the Cre recombinase in cholinergic, glutamatergic or GABAergic neurons. Inhibition of GABAergic neurons was sufficient to mirror full inhibition of the LDTg. Inhibition of cholinergic or glutamatergic failed to prevent stress-induced freezing.

In order to delineate the brain circuits in which LDTg GABAergic neurons are embedded to modulate freezing behaviors, we combined immunohistochemistry approaches with viral injections of fluorescent tracers. Once the functional role of those projections is established, these results will help us redesign the map of defensive behaviors circuitry in the brain by defining where LDTg belongs on this map.

P-47

The role of the chemokine CCL17 in brain development and behavior

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Labex Signalife

The development of the nervous system is a tightly orchestrated process where small changes during the prenatal period can lead to neurodevelopmental disorders (NDDs), such as autism spectrum disorder (ASD) or attention deficit and hyperactivity disorder (ADHD). NDDs are multifactorial disorders causing lifelong disabilities and major social concern. An important risk factor for NDD, particularly ASD, is maternal infection during pregnancy. 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 including social interaction and communication deficits and motor abnormalities. Moreover, ASD is associated with altered profiles of immune markers such as cytokines and chemokines. In this maternal immune activation (MIA) model, neutralisation of specific pro-

inflammatory cytokines in pregnant mothers alleviates behavioural alterations in the progeny, further demonstrating their critical role in NDD.

While the role of specific pro-inflammatory cytokines in NDD has been clearly demonstrated, little is known on the possible role of chemokines. We recently identified the chemokine CCL17 to be elevated in the serum of children with emotional difficulties and hyperactivity, based on a longitudinal study performed on 900 5-year old children. Moreover, mice lacking CCR4, the receptor for CCL17, exhibit behavioural abnormalities, suggesting that altered CCL17 levels could be involved in NDD. We therefore investigated the role of CCL17 in brain development and function in the murine MIA model of NDD.

The MIA model was obtained by injecting poly(I:C) to pregnant dams at embryonic day 10.5. We further studied the progenyâ€™s behaviour. 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.

To further characterise the role played by CCL17 in the development or maintenance of behavioural impairment, we plan to neutralise CCL17 either in pregnant dams or young pups to alleviate abnormal neurodevelopment in MIA mice.

P-48

Regulation ot T cell activation by Fas receptor

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Two signals are required for full activation of T cells : (i) one generated from the interaction between the T cell receptor (TCR) and a peptide, presented by antigen-presenting cells (APCs) via major histocompatibility complex (MHC) class I or II molecules, (ii) one initiated upon the interaction between a T cell costimulatory receptor and its ligand on the APC, which can positively or negatively influence T cell activation. Fas/CD95, a member of the TNFR superfamily is a crucial regulator of T cell homeostasis. Apart from its pro-apoptotic role in different cell types including activated T lymphocytes, Fas can also rise to diverse survival signals. In T cells, Fas has been reported affecting proliferation, differentiation and migration processes as well as cytokine production. Importantly, its modulatory role has been reported in numerous T cell activation studies, although neither its exact importance nor the molecular mechanisms underlying this involvement were so far elucidated.

The aim of my PhD project is to (i) clarify the role of Fas on T cell activation by TCR and (ii) Decipher the molecular basis of this regulation by exploiting both biochemical and biophysical techniques.

Our first results described for the first time with a native membrane bound Fas ligand and an antigen specific TCR, a costimulatory role of Fas for the TCR activation . We indeed show that the 3A9 CD4+ hybridoma cells which display a TCR specific for HEL peptide bound to MHC class II I-Ak molecule expressed on a APC cell, increase their IL2 secretion and their CD69 expression when Fas Ligand is present on the APC. At the molecular level, this coactivation is validated by increased phosphorylation of TCR early signaling molecules such as CD3, ZAP-70 and LAT. We confirmed these results in human cell activated by coated anti-CD3/CD28 and soluble anti-Fas antibodies.

As Fas coactivation functions appear to be mediated by Fas ability to interfere with the very early steps of TCR signaling, we will investigate this cross talk by studying more specifically Fas and TCR membrane organization

and dynamics, upon T cell and FasL activation by combining biochemical and biophysical approaches. Considering the main clinical advances obtained in cancer immunotherapy targeting TCR coactivation, we will summarize our recent findings with a focus on the costimulatory capacity of Fas in T cells and their potential implications in cancer immunotherapy.

P-49

Polyunsaturated phospholipids facilitate the closure of transendothelial cell macroaperture induced by bacterial toxin

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iBV

The unsaturation of acyl chains in phospholipids affects the property of cellular membranes. In our previous study, we reported polyunsaturated phospholipids (PUPL) facilities membrane deformation and fission. However, the function of PUPL in cells is poorly understood. Here, our study focuses on the impact of PUPL on bacterial infection, a newly described mechanism of Staphylococcus aureus disseminates through the endothelium barrier. The secretion of bacterial toxin induces the opening of transcellular holes in endothelial cells, termed TransEndothelial cell Macroaperture (TEMs). The process involves large-scale membrane deformation, fission, and fusion. We found PUPL accelerated the speed of TEM closure; hence reduce the size of TEM. The study brings out the possibility that PUPL helps cells defense against bacteria invasion.

P-50

The Response heterogeneity of clonal cancer cells to death ligands

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The objective of my thesis is to study the response heterogeneity of clonal cancer cells to death ligands. First, we are studying the Heterogeneity in Drug Sensitivity (vs. Therapeutic Resistance) To determine the molecular origins of response heterogeneity to TRAIL, a death ligand drug. I'm using for this a single cell method to be able to discriminate individually each cell resistant and sensitive in population. HeLa cells are stably expressing a probe to monitor the caspase 8 activity, based on FRET principle. During Apoptosis induction, we follow the caspase 8 activity in each of clonal cancer cell by Live Cell Microscopy. We recently found we were able to predict the cell fate of a cell by the caspase 8 activity after only 50 minutes of treatment. Because it's known that cells are reprogramming during time of treatment, we performed a RNAseq on cells predict to be sensitive or resistant to found molecular origins of resistance to TRAIL treatment. We are currently validating candidate's genes with promising preliminary results. Secondly, we want to determine the role of p62 and RIP3K in cell death modality balance in response to pro-apoptotic drugs; Developing 4-color live cell microscopy. We discovered heterogeneity of response was more than resistant versus sensitive. We observed by Live Cell Microscopy differences in Cell death modalities during cell death induction by TRAIL, clonal cells dying by different way under only one treatment. We decide to focus on Apoptosis, a non-immunogenic type of cell death normally induced by TRAIL treatment, and Necroptosis, an highly immunogenic type of cell death recently discovered. We investigate the role of two molecules, P62 and RIPK3, two gene candidate involved in this both pathway to explain the balance between this two type of cell death.