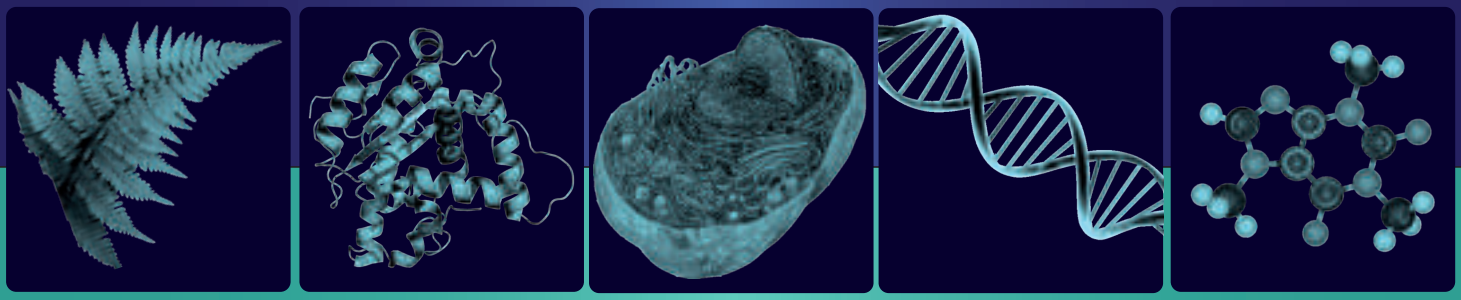
JEDNs

15th JEDNS85 2015

3 & 4 SEPTEMBER 2015
FACULTY OF SCIENCES
NICE

# JOURNEES DE L'ECOLE DOCTORALE85 DE NICE



**FOR PROGRAM DETAILS AND REGISTRATION PLEASE VISIT: WWW.JEDNS-BIO.FR**

# SCIENTIFIC PROGRAM

## INVITED SPEAKERS:

**LORENZO GALLUZZI,** INSITUT DE CANCEROLOGIE GUSTAVE ROUSSY, PARIS.
**MARIE MIROUZE,** INSTITUT DE RECHERCHE POUR LE DEVELOPPEMENT, MONTPELLIER.
**BART STAELS,** INSTITUT PASTEUR DE LILLE, LILLE.

ED85 Sciences Vie Santé

Université Nice Sophia Antipolis

Membre de UNIVERSITÉ CÔTE D'AZUR

PEPROTECH

CliniSciences

PromoCell

Nikon

EUROMEDEX

Promega

# CONTENTS

# OUR SPONSORS

Université Nice Sophia Antipolis

ED 85 Sciences Vie Santé

VILLE DE NICE
www.nice.fr

PEPROTECH
Manufacturer of Quality Cytokine Products

CliniSciences
www.clinisciences.com

PromoCell

Nikon

EUROMEDEX
The Experts in Health

Promega

VWR

# OUR GUESTS

## Dr Lorenzo GALLUZZI

Post-doctoral fellow in the « Apoptosis, cancer and immunity » team (group leader: Prof. Guido Kroemer) in Gustave Roussy Cancer Campus, Grand Paris.

## Dr Marie MIROUZE

Researcher in the team « RICE » (Rice, Interspecies Comparison & Evolution, group leader: Laurence Albar) in DIADE research unit, in the Institute of Research for Development, Marseille. She is based in Université de Perpignan Via Domitia.

## Dr Bart STAELS

Leader of the team « Nuclear receptors in metabolic syndrome », and Director of the research unit « Nuclear receptors, cardiovascular diseases and diabetes » in the Pasteur Institute, Lille.

# ORGANIZING TEAM

**Ramona GALANTONU** - 2nd year Ph.D. student

Team « Retrotransposons and genome plasticity »
Institut de recherche sur le cancer et le vieillissement (IRCAN, Nice)

**Maude GIROUD** - 3rd year Ph.D. student

President of the Association of PhD students in Biology of Nice
Team « Cellular and molecular regulation of fat mass »
Institut de Biologie de Valrose (IBV, Nice)

**Ophélia LE THUC** - 3rd year Ph.D. student

Team « GENE - Genomics and Evolution in NeuroEndocrinology »
Institut de Pharmacologie Moléculaire et Cellulaire (IPMC, Sophia-Antipolis)

**Lauriane MASSARDIER** - 2nd year Ph.D. student

Team « Theoretical Ecology and its Applications to Protected Environment Agrosystems »
Institut Sophia Agrobiotech (ISA, Sophia Antipolis)

**Clémence MEDINA** - 2nd year Ph.D. student

Team « Plant-Nematode Interactions »
Institut Sophia Agrobiotech (ISA, Sophia Antipolis)

**Elodie NAESSENS** - 2nd year Ph.D. student

Teams « Plant-Oomycete Interactions » and « Insect Defence »
Institut Sophia Agrobiotech (ISA, Sophia-Antipolis)

**Elodie VILLA** - 2nd year Ph.D. student

Treasurer of the Association of PhD students in Biology of Nice
Team « Metabolic control of cell death »
Centre Méditerranéen de Médecine Moléculaire (C3M, Nice)

**Bastien VERGONI** - 1st year Ph.D. student

Secretary of the Association of PhD students in Biology of Nice
Team « Molecular and cellular pathophysiology of obesity and diabetes »
Centre Méditerranéen de Médecine Moléculaire (C3M, Nice)

# DETAILED PROGRAM

## Thursday, September 3rd 2015

**07:30-08:00 Registration**

**08:00-08:15 Opening speech, Dr Thomas LAMONERIE, director of the Doctoral School**

**08:15-09:30 Oral Communications - session 1**

1. **Nedra TEKAYA**
   *Identification and characterization of novel protein complex regulating microRNA targeting* ..... *p.21*
2. **Grégoire SAVARY**
   *miR-214-3p, a new fibromiR involved in the pathogenesis of idiopathic pulmonary fibrosis* ..... *p.22*
3. **Laetitia BERG ALONSO**
   *Mitofusin-2 gene and mitochondrial disorders* ..... *p.23*
4. **Olivier MERCEY**
   *Genomic mechanisms controlling multiciliated cell differentiation during airway epithelium regeneration* ..... *p.24*

**09:30-10:30 Oral Communications - session 2**

1. **Alix DUBOIS**
   *LAMP2 deficiency in MDS/AML cells is associated with resistance to Azacytidine that can be circumvented by lysosomal and autophagy inhibitors* ..... *p.25*
2. **Christophe RAVAUD**
   *Heterogeneous alteration of the distinct adipose tissues by HAART therapy: Molecular mechanisms, modelling and importance of their origin and function* ..... *p.26*
3. **Delphine BAUDOUY**
   *Role of Wilms' tumor suppressor (Wt1) in cardiac angiogenesis and function after myocardial infarction in adult mice* ..... *p.27*
4. **Daniel HAMAOUI**
   *Optineurin's role in the HACE1/Rac1 axis* ..... *p.28*
5. **Sonia BOULAKIRBA**
   *Arf6 exchange factor EFA6 and endophilin directly interact at the plasma membrane to control clathrin-mediated endocytosis* ..... *p.29*

**10:30-11:00 Coffee break**

**11:00-11:45 Oral Communications - session 3**

1. **Thibault MOREL-JOURNEL**
   *Introduction strategies in spatially structured environments* ..... *p.30*
2. **Marie LAROUSSE**
   *Microbiota diversity associated with root infection of Solanum lycopersicum by Phytophthora parasitica* ..... *p.31*
3. **Rihem MOUJAHED**
   *Consequence of dual biotic stresses on plant volatile emission and recruitment of egg parasitoids* ..... *p.32*

**11:45-12:00 « My Poster in 30 seconds » - session 1**

**12:00-13:00 Keynote Lecture. Dr Bart STAELS**

*Circadian control of metabolism: Rev-erbs as potential therapeutic targets.*

**13:00-13:30 Lunch break**

**13:30-15:00 Poster session + Workshop « Career opportunities in a biotechnology company »**

**15:00-16:00 Keynote Lecture. Dr Marie MIROUZE**

*The epigenome : a guardian of the plant mobilome?*

**16:00-16:30 Coffee break**

**16:30-17:30 Oral Communications - session 4**

1. **Joséphine PARISOT**
   *The transcription factor COUP-TF1 regulates hippocampus development and migration of the dentate gyrus granule cells* ..... *p.33*
2. **Jennifer LAVIGNE**
   *The pathological state before the onset of Epilepsy* ..... *p.34*
3. **Miled BOUROUROU**
   *Alpha-linolenic acid supplementation impoves motor and cognitive functions in a mice model of stroke* ..... *p.35*
4. **Emmanuelle DOR**
   *Childhood Onset Schizophrenia: Epidemiology, Clinical and Neurocognitive Exploration, Relationship With the Autism Spectrum Disorders* ..... *p.36*

**17:30-19:00 Drinks**

**19:00-22:00 Dinner**

# Friday, September 4th 2015

**08:00-08:30 Registration**

**08:30-09:30 Oral Communications - session 5**

1. **Yann CORMERAIS**
   *Dissecting the pro-tumoral role of the CD98/LAT1 complex* ..... *p.37*
2. **Emilien GARCIA**
   *The SYK tyrosine kinase negatively regulates β1 integrin function and migration of melanoma cells* ..... *p.38*
3. **Florian ROUAUD**
   *Implication of E2F1 transcription factor in melanoma* ..... *p.39*
4. **Sahar AL-QARAGHULI**
   *Role of Xeroderma pigmentosum fibroblasts in squamous cell carcinoma cell invasion* ..... *p.40*
5. **Thomas GOIRAN**
   *Study of PINK1 in Human Tumor Development* ..... *p.41*

**09:30-10:30 Keynote Lecture. Dr Lorenzo GALLUZZI**

*Unsaturated fatty acids induce non-canonical autophagy*

**10:30-11:00 Coffee break**

**11:00-12:00 Round Table - UNICEPRO**

**12:00-12:15 « My Poster in 30 seconds » - session 2**

**12:15-13:00 Lunch break**

**13:00-14:30 Poster session**

**14:30-15:45 Oral Communications - session 6**

1. **Rayane GHANDOUR**
   *Regulation of the conversion of white to brown adipocytes by arachidonic acid metabolic pathway* ..... *p.42*
2. **Noémie DRUELLE**
   *Pancreatic somatostatin-expressing cells: An untapped source for beta-cell regeneration?* ..... *p.43*

3. **Claudine MORATAL**
   *Regulation of Adipogenic Lineage in Healthy and Dystrophic Human Muscles* ..... *p.44*

4. **Nicolas FORCIOLI-CONTI**
   *Role of primary cilium during adipocyte differentiation* ..... *p.45*

5. **Ophélia LE THUC**
   *A new role for Monocyte chemoattractant protein 1/CCL2 in promoting weight loss through inhibition of melanin-concentrating hormone-expressing neurons.* ..... *p.46*

6. **Maude GIROUD**
   *Targeting miR-125b-5p to fight obesity via browning of white adipose tissue.* ..... *p.47*

## 15:45-16:45 Oral Communications - session 7

1. **Emilie BOONE**
   *The study of Dilp8, a new hormone coordinating organ growth* ..... *p.48*

2. **Fariba Jian MOTAMEDI**
   *WT1 controls antagonistic FGF and BMP-pSMAD pathways in early renal progenitors* ..... *p.49*

3. **Guillaume DOLLA**
   *A PLA2R1 epitope study in Membranous Nephropathy : diagnostic and prognostic significance* ..... *p.50*

4. **Elia MAGRINELLI**
   *The cortical patterning gene COUP-TFI regulates major components of neuronal activity during development* ..... *p.51*

5. **Jonathan PINI**
   *Human induced pluripotent stem cells from Andersen's syndrome patients: Implication of the Kir2.1 potassium channel in bone morphogenesis* ..... *p.52*

## 16:45-17:15 JEDNs Awards

# POSTER SESSIONS

## Thursday, September 3rd 2015

# POSTER SESSIONS

## Friday, September 4th 2015

# ORAL COMMUNICATIONS

## *Abstracts*

Oral Communications - Session 1/OC n°1

# Identification and characterization of novel protein complex regulating microRNA targeting

*Nedra Tekaya*, Silvia Bottini, Laure-Emmanuelle Zaragozi, Raphaël Mategot, Stéphane Audebert, Emmanuela Repetto, Zoheir Hizir, Pascal Barbry, Michele Trabucchi*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

Functional interaction between RNA-binding proteins (RBP) and miRNAs by a combinatorial code of competition and synergy to binding sites is a fundamental principle of post-transcription gene expression control. In agreement with this principle, some RBPs that associate with miRNA-target mRNAs can interfere with miRISC (miRNA-induced silencing complex) binding and function to either inhibit or enhance the miRNA mode of action in a dynamic fashion. This leads to the concept of a sequence microenvironment surrounding the miRNA-binding sites that plays an important role in miRNA activity. During my PhD, I have discovered by mass-spectrometry that the multifunctional RBPs Splicing factor proline/glutamine-rich (Sfpq) binds to the sequence surrounding the miRNA-binding sites. Indeed, I found that Sfpq associates with Ago2 and different miRNAs, including let-7a, in both cytoplasm and nucleus. Importantly, Sfpq co-localizes with the processing bodies (P-bodies), a cytoplasmic organelle, in which the miRNA-mediated RNA degradation takes place. Therefore, these data indicate that Sfpq binds to the sequence surrounding miRNA-binding sites raising the possibility that could regulate the mechanism of silencing mediated by miRNAs in the P-bodies and may also be involved in modulating miRNA nuclear activity. Functional investigation of Sfpq role has been done by microarray coupled with bioinformatic analysis to study the expression of Ago2-associated miRNAs in P19 stem cells, we found a significant enrichment of the putative Sfpq-binding sites nearby the binding sites of endogenous miRNAs, as well as, for the target mRNAs of the ectopically expressed let-7a. About 80% of the let-7a downregulation of cognate mRNAs was rescued by transfecting si-Sfpq. Our datas uncover the role of new RBP: Sfpq that modulates the miRNA function/accessibility by acting in the sequence surrounding the miRNA-binding sites.

Oral Communications - Session 1/OC n°2

# miR-214-3p, a new fibromiR involved in the pathogenesis of idiopathic pulmonary fibrosis

*Grégoire Savary*, Matthieu Buscot, Edmone Dewaeles, Imène Sarah Henaoui, Stéphanie Quarré, Elisabeth Courcot, Christelle Cauffiez, Pascal Barbry, Michael Perrais, Nicolas Pottier and Bernard Mari*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Recent evidence has unveiled a critical role of miRNAs in the pathogenesis of Idiopathic Pulmonary Fibrosis (IPF). In particular, we established miR-199a-5p as a major regulator of lung fibroblast/myofibroblasts differentiation by targeting CAV1, a key player in TGFβ signaling (Henaoui, IS. et al. Plos Genetics 2013, 9:2). Interestingly, miR-199a-5p is transcribed from the miR-199a/214 gene cluster to generate a primary transcript DNM3os (Dynamin 3 Opposite Strand). TGFβ stimulation of lung fibroblasts enhanced the expression of DNM3os and of the 3 mature miRNAs (miR-199a-3p, miR-199a-5p and miR-214-3p), suggesting a transcriptional regulation of the whole cluster. In order to establish the role of this cluster in the pathogenesis of IPF, we focused on the contribution of miR-214 to fibrotic mechanisms. First, we demonstrated that miR-214 overexpression was sufficient to promote lung fibroblast differentiation into myofibroblasts and protect cells from Fas-mediated cell death. Transcriptomic analysis of miR-214 overexpressing lung fibroblasts led us to identify several miR-214 targets associated with fibrogenesis: i) GSK3β, the main inhibitor of the Wnt/β catenin pathway, a non canonical TGFβ pathway, involved in the differentiation of fibroblast into myofibroblast ; ii) PGE2, a well known antifibrotic and anti-apoptotic prostaglandin, which is crucial for lung epithelial regeneration through the PGE2/COX-2/HGF axis. Overall, we show here that the miR-199a/214 cluster functions as a key regulator of both canonic and non-canonic TGFβ pathways and as a critical actor of myofibroblast differentiation and epithelial-mesenchymal interactions. The aberrant expression of this cluster in IPF patients and in other fibrotic diseases including Duchenne Muscular Distrophy, suggest that it could represent a new attractive therapeutic target for these devastating diseases.

# Mitofusin-2 gene and mitochondrial disorders

*Laetitia Berg Alonso*, Cécile Rouzier, Sylvie Bannwarth, Gaelle Augé, Emmanuelle Genin, Annabelle Chaussenot, Konstantina Fragaki, Françoise Lespinasse, Véronique Paquis-Flucklinger*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

Mitochondria are essential organelles that generate energy from oxidative phosphorylation in eukaryotic cells and play a role in various cellular processes. These are believed to derive from endosymbiosis of bacteria, and contain their own genome : the mitochondrial DNA (mtDNA) within the matrix. Mitochondrial disorders are be caused by mutations in mtDNA or mainly in nuclear genes that encode for mitochondrial components. We focus our interest on mitochondrial diseases with mtDNA instability, characterized by reduction of mtDNA copy number (depletion) or by accumulation by mtDNA multiple deletions in post-mitotic tissues. Nuclear genes responsible for mtDNA instability disorders mainly fall into three categories: genes encoding proteins directly involved in mtDNA replication, in the mitochondrial nucleotide pool maintenance, and genes encoding proteins, such as OPA1 or Mitofusin-2 (Mfn2), responsible for mitochondrial fusion. Mfn2 is a mitochondrial GTPase of the dynamin family that participates in mitochondrial dynamics, plays a crucial role in fusion of outer mitochondrial membranes and contributes to the maintenance of the mitochondrial network. MFN2 mutations are responsible for Charcot Marie Tooth type 2 disease (CMT2A), a peripheral neuropathy without mtDNA instability. We have identified a missense mutation (p.D210V), located in the GTPase domain of Mfn2, in a large family presenting with a complex neurological phenotype including optic atrophy and mtDNA deletions in muscle. We want to understand mechanisms responsible for this mtDNA instability by comparing the impact of this MFN2 mutation and the one from a classical deleterious variant (p.A166T) causing CMT2A on mitochondrial functions to define the links between mitochondrial fusion and instability of the mitochondrial genome. Studies of patient fibroblasts show that only those carrying the D210V mutation present a fusion defect that could explain the severe phenotype that we observed.

# Genomic mechanisms controlling multiciliated cell differentiation during airway epithelium regeneration

*Olivier Mercey*, Benoït Chevalier, Marie Cibois, Anna Adamiok, Guillaume Luxardi, Laure-Emmanuelle Zaragosi, Andrea Pasini, Laurent Kodjabachian, Valérie Julia, Pascal Barbry, Brice Marcet*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

The airway epithelium lining the surface of the respiratory tract is the first line of defense which protects the organism against external aggressions (inhaled toxic particles, pathogens, allergens…). It is constituted by basal cells, goblet cells and a majority of MultiCiliated Cells (MCCs) which project hundreds of motile cilia at their apical surface. Their coordinated beating is crucial to orchestrate the mucociliary clearance essential for airway cleansing. In chronic respiratory diseases, tissue remodeling is accompanied with global loss of MCCs leading to reduced mucociliary clearance.

In this context, our laboratory aims at understanding molecular mechanisms allowing MCC progenitors to develop motile cilia and to become mature MCCs. Using two in vitro models of vertebrate mucociliary epithelium (from human and murine airways) and two in vivo models (Xenopus embryonic epidermis, and an in vivo model of asthma in mouse), we elucidated the successive mechanisms controlling MCC differentiation. First, we showed that the BMP signal modulated at an early step the Notch pathway to control vertebrate MCC differentiation. Then, we identified microRNAs of the miR-34/449 family as key and conserved regulators in this process. miR-34/449 allow a definitive cell cycle exit by repressing cell cycle related genes. They promote differentiation through the direct repression of the Notch pathway. Furthermore, they control the apical reorganization of the actin network, a pre-requisite for motile cilia formation, by directly repressing the small GTPase R-Ras. Finally, we detected global changes in DNA methylation status during human airway epithelium differentiation that could drive the multiciliated program.

Our study establishes miR-34/449 as central actors controlling different key pathways in MCC differentiation and opens the way for the development of new therapeutic approaches for treating chronic airway diseases associated with ciliary defects.

Oral Communications - Session 2/OC n°1

# LAMP2 deficiency in MDS/AML cells is associated with resistance to Azacytidine that can be circumvented by lysosomal and autophagy inhibitors

*Alix DUBOIS*, Clémence GINET, Thomas CLUZEAU, Mohamed Amine HAMOUDA, François ORANGE, Sandra LACAS-GERVAIS, Valentine RICHEZ, Jean-Michel KARSENTI, Arnaud JACQUEL, Sandrine MARCHETTI, Frederic LUCIANO, Pierre Simon RORLISCH, Guillaume ROBERT# and Patrick AUBERGER#*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

CONFIDENTIAL

# Heterogeneous alteration of the distinct adipose tissues by HAART therapy: Molecular mechanisms, modelling and importance of their origin and function

*Christophe Ravaud*, Stéphane Azoulay, Anne-Laure Hafner, David Estève, Anne Bouloumié, Christian Dani and Annie Ladoux*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

AIDS has been a devastating disease with more than 25 million deaths worldwide since the beginning of the 80's and 34 million people are still infected with HIV as no efficient vaccination exists. The Highly Active AntiRetroviral Therapy (HAART) has considerably improved life expectancy and decreased significantly the viral charge of AIDS patients, thus reducing the HIV propagation. This therapy is composed of HIV Protease Inhibitors (PI), such as Lopinavir (LPV) or Darunavir (DRV) and Nucleoside Reverse Transcriptase Inhibitors (NRTI). However, its use has been hindered by many metabolic adverse side effects such as lipodystrophy apparition. Lot of studies investigated the effects of PI on adipose differentiation but few of them reported their impact on the self-renewal of adipocyte progenitors (APs) and on adipocytes from different embryonic origins. In the present study, we analysed the effects of LPV, one of the most efficient and prescribed drugs in developed countries, despite adverse effects reported and DRV, a last generation of PI that seems to be better tolerated by patients, on self-renewal and differentiation of APs from distinct fat depots such as chin or knee known to generate different types of adipocytes. We first characterized IER3 as an important gene involved in APs self-renewal. Its expression is dependent on the microenvironment and is increased in over-proliferative tissues such as fat pads of obese patients. LPV dramatically impaired APs self-renewal and decreased IER3 expression while DRV had no significant impact. Moreover, while LPV blunted adipose differentiation, DRV did not impact this process, whatever APs were used. In addition DRV did not induce insulin resistance or ER stress in adipocytes. All-together our results indicate that DRV produced less alteration than LPV on both APs and adipocytes, respecting then adipose tissue integrity. Thus, DRV treatment should improve the quality of life of AIDS patients receiving HAART therapy.

Oral Communications - Session 2/OC n°3

# Role of Wilms' tumor suppressor (Wt1) in cardiac angiogenesis and function after myocardial infarction in adult mice

*D. Baudouy*, A. Vukolic, P. Lopez, N. Wagner, K.D. Wagner*

**Institut for Research on Cancer and Aging, CNRS UMR 7284-INSERM U1081-Université Nice Sophia Antipolis, Nice*

Myocardial infarction (MI) is a major cause of mortality. During embryonic development, Wt1 (Wilms' tumor suppressor) controls epithelial to mesenchymal transition in epicardial cells, a key step in cardiac angiogenesis. After MI, Wt1 is re-expressed in coronary vessels and colocalised with angiogenic factors. We postulated that Wt1 was required for cardiac repair after MI. We sought to assess the importance of Wt1 in cardiac vascularisation and function after MI and to identify Wt1–dependent genes. We used a model of conditional vascular expression of Wt1 in adult mice (Tie2-Cre-ERT2;Wt1loxP+/+). We mimicked MI by coronary artery ligation. We compared mice with Wt1 KO (tamoxifen injection), mice with normal Wt1 expression (vehicle injection), and a group of mice to control for Cre and Tamoxifen effects (Tie2-Cre-ERT2 + Tamoxifen injection). We performed an echocardiography before and after MI (acute, reparation and chronic phases) and histological analyses on heart sections. Molecular analyses determined RNA differential expression between our groups. We showed that Wt1 KO did not affect cardiac function before MI. At the acute phase of MI, it caused cardiac dilation, increased MI size, cardiomyocyte hypertrophy, cardiac fibrosis, and a decrease in vascular density. With transfection experiments, we identified angiopoietin-like 3 and endothelin 1 as Wt1 target genes. Wt1 was necessary for cardiac repair after MI: in Wt1 KO mice, we observed an increase in cardiac dilation and fibrosis, in cardiomyocyte and MI size, and in apoptosis. In parallel, those mice showed lower contractility and vascular density. A triple transgenic mouse line (Wt1-GFPki;Tie2-Cre-ERT2;Wt1loxP+/-) will allow us to identify genes involved in cardiac repair after MI (CHIP-sequencing on GFP positive cells). Understanding the mechanisms of cardiac repair after MI, including Wt1-mediated neoangiogenesis, is of high importance to find novel treatment strategies after MI.

Oral Communications - Session 2/OC n°4

# Optineurin's role in the HACE1/Rac1 axis

*D. Hamaoui*, A. Doye, E. Andrio, E. Lemichez, A. Mettouchi*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

Rac1 is a G protein from the Rho GTPase family that controls cellular processes such as cell adhesion, proliferation and inflammation. Therefore mutations, gene amplification or deregulation of Rac1 are commonly associated with disease. We recently identified that the E3 ubiquitin ligase HACE1 catalyzes the ubiquitylation of active Rac1 to downregulate its activity. HACE1 is considered a tumor suppressor and little is understood about its functions. To better understand the HACE1/Rac1 pathway we performed a whole genome 2-hybrid screen assay to determine new partners of HACE1. We found that Optineurin (OPTN) is the main interactant of HACE1, and the subject of my thesis is to understand OPTN's role in the HACE1/Rac1 pathway. We started by analyzing whether OPTN influences HACE1's targeting of Rac1, and we found that OPTN seems to be essential for HACE1's ubiquitylation and interaction with Rac1. We then studied the cellular context in which OPTN controls Rac1 ubiquitylation. We found that OPTN is localized in focal adhesion structures. Using loss of function experiments we show that OPTN downregulates Focal adhesion (FA) maturation. More over, we showed that the loss of OPTN resulted in an increase in Rac1 activation by integrins (ITG) α5ß1 that leads to higher cycline D1 translation, a known oncogene. We also demonstrated that this increase in translation is due to an over-activation of the regulator of cell anabolism mTORC1. FAs are in the interface between the extracellular matrix (ECM) and cell behavior. It has been shown that cells respond and control the stiffness of the ECM, and abnormal ECM stiffness can overdrive integrin mediated cell growth in cancerous tissues. In the last part of our study we demonstrate that the loss OPTN yields an increase in cell division in response to ECM stiffness increase, which in turn is due to an over-activation of the ITGα5ß1/Rac1/mTORC1/CyclineD1 signaling pathway. OPTN therefore acts as a novel sensor of ECM stiffness.

# Arf6 exchange factor EFA6 and endophilin directly interact at the plasma membrane to control clathrin-mediated endocytosis

*S. Boulakirba*, E. Macia, M. Partisani, S. Lacas-Gervais, F. Brau, F. Luton, and M. Franco*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Members of the Arf family of small G proteins are involved in membrane traffic and organelle structure. They control the recruitment of coat proteins, and modulate the structure of actin filaments and the lipid composition of membranes. The Arf6 isoform and its specific exchange factor EFA6 are known to regulate the endocytic pathway of many different receptors. In order to determine the molecular mechanism of the EFA6/Arf6 function in vesicular transport we searched for new EFA6 partners. In a two hybrid screening using the catalytic Sec7 domain as a bait, we identified endophilin as a new partner of EFA6. Endophilin contains an N-Bar domain responsible for membrane bending and an SH3 domain responsible for the recruitment of dynamin and synaptojanin, two proteins involved respectively in fission and uncoating of clathrin-coated vesicles. Using purified proteins, we confirmed the direct interaction, and identified the N-Bar domain as the binding motif to EFA6. We showed that endophilin stimulates the catalytic activity of EFA6 on Arf6. In addition, we observed that the Sec7 domain competes with flat but not with highly curved lipid membranes to bind the N-Bar. In cells, expression of EFA6 recruits endophilin to EFA6-positive plasma membrane ruffles, while expression of endophilin rescues the EFA6-mediated inhibition of transferrin internalization. Overall, our results support a model whereby EFA6 recruits endophilin on flat areas of the plasma membrane to control Arf6 activation and clathrin-mediated endocytosis.

Oral Communications - Session 3/OC n°1

# Introduction strategies in spatially structured environments

*T. Morel-Journel*, E. Vercken, L. Mailleret*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Biological invasion is a sequential process comprising several stages, from the arrival of an exotic species in a new site to the spread of this species across larges areas. The establishment, a major stage of invasion, is reached when the introduced individuals are able to survive in their new environment and reproduce locally. Yet, most introduced populations never reach this stage and go extinct quickly. Understanding the main factors underlying establishment success is thus critical to prevent unintentional introductions and facilitate the intentional ones. The area where individuals are introduced is hardly homogeneous. It is a landscape, which usually comprises several habitat patches more or less favorable to the introduced species. By affecting the dispersal, survival and reproduction of the individuals, the spatial structure of the landscape might impact the establishment rate. My thesis subject focuses on understanding the impact of landscape structure on the establishment of introduced populations. To do so, we developed models of population dynamics in a spatial context, describing individual migration and reproduction according to parameters related to landscape structure. Predictions about the impact of different landscape features on establishment could then be tested by experiment. To do so, we performed artificial introductions of hymenopteran parasitoids in laboratory microcosms and monitored their invasion over several generations. The recorded population dynamics would then inform us on the veracity of our theoretical predictions in the context of an actual invasion.

Oral Communications - Session 3/OC n°2

# Microbiota diversity associated with root infection of *Solanum lycopersicum* by *Phytophthora parasitica*

*Marie Larousse*, Corinne Rancurel, Mura Catherine, Marc Bardin, Xavier Nesme, Eric Galiana*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

The interactions between a plant pathogen and the host surface resident microbiota are critical to disease outcome. These interactions shape the distribution, the density and the genetic diversity of inocula. However for most plant pathogens how each of these interactions acts on disease as a single one or as a member of a functional network remains to be specified. In this work , the interactions between the prokaryotic microbiota of *Solanum lycopersicum* rhizosphere and *P. parasitica* were explored. Using first a metagenomic approach two microbial metagenomes derived from a soil of a tomato greenhouse were defined and compared: M1 which corresponds to the sub-rhizospheric microbiota able to colonize the roots of axenic tomato seedlings; M2, the sub-microbiota able to colonize the tomato seedling roots previously coated with *P. parasitica* monospecific biofilm. 16S RNA gene sequencing revealed that at the Phylum level, M1 and M2 differ in abundance of some taxa but not in microbial diversity. On the other hand, a representative collection of microorganisms from M2 were obtained through in vitro selection on a medium prepared from *P. parasitica* extract. One thousand and two hundred isolates were screened for impact on *P. parasitica* growth; 1.2% and 9.8% of isolates had a helper or inhibitor growth effect mediated by secreted metabolites. From these isolates, GFP expressing strains were generated and found to be associated with *P. parasitica* biofilm in which they modulate oomycete gene expression for mucin-like proteins or effectors that promote successful infection. Taken together these results indicate that at the early step of plant infection a sub-microbiota is physically associated with the oomycete and may interfere with its growth and virulence.

# Consequence of dual biotic stresses on plant volatile emission and recruitment of egg parasitoids

*Rihem Moujahed*, Francesca Frati , Antonino Cusumano, Gianandrea Salerno , Eric Conti , Ezio Peri and Stefano Colazza*

**Università degli Studi di Palermo, Palermo, Italy*

Plants respond to insect oviposition by emission of oviposition-induced plant volatiles (OIPVs) which can recruit egg parasitoids of the attacking herbivore. To date, studies demonstrating egg parasitoid attraction to OIPVs have been carried out in tritrophic systems. Less attention has been given to plants experiencing multiple attacks by host and non-host herbivores that potentially could interfere with the recruitment of egg parasitoids as a result of modifications to the OIPV blend. Egg parasitoid attraction could also be influenced by the temporal dynamics of multiple infestations, when the same non-host herbivore damages different organs of the same plant species. In this scenario we investigated the responses of egg parasitoids to feeding and oviposition damage using a model system consisting of *Vicia faba*, the above-ground insect herbivore *Nezara viridula*, the above-and below- ground insect herbivore *Sitona lineatus*, and *Trissolcus basalis*, an natural enemy of *N. viridula*. The response of wasp females to *V. faba* volatiles was investigated in a Y-tube olfactometer testing plants that were infested with insects above-ground, below-ground, and both above and below ground. The emission of plant volatiles in response to above-below ground attacks were also chemically analyzed by Gas chromatography-Mass Spectrometry. The results showed that the non-host *S.lineatus* disrupts wasp attraction toward plant volatiles induced by the host *N. viridula*. Chemical analysis indicated differences between volatiles emitted by *V. faba* plants in response to *N. viridula* feeding and oviposition and volatile emitted as consequence of dual insect infestation.

Oral Communications - Session 4/OC n°1

# The transcription factor COUP-TF1 regulates hippocampus development and migration of the dentate gyrus granule cells

*Joséphine Parisot*, Gemma Flore and Michèle Studer*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

The hippocampus is a major component of the mammalian brain. It belongs to the archicortical domain, the more ancient portion of the telencephalon, and plays an important role in memory, learning, and spatial navigation. The hippocampal formation comprises two cytoarchitectonically distinct regions : the hippocampus proper and the dentate gyrus (DG), showing a three-layered appearance. The DG is the primary gateway for input information into the hippocampus and its development begins during gestation on Embryonic day 14 (E14.5) in mouse. Our gene of interest, the nuclear receptor COUP-TF1 has been shown to act as a strong transcriptional regulator of the developing neocortex, having, among others, key roles in arealization and lamination. Yet, little is known about its involvement outside the neocortex, in the hippocampus. The aim of our research is therefore to understand the function of COUP-TF1 in the developing hippocampus, and specifically in cell differentiation and migration. We have shown that COUP-TF1 is expressed in both proliferating and differentiating neural progenitors in the hippocampus, and is strongly expressed in the dentate gyrus neuroepithelium. In mouse brains in which COUP-TF1 is inactivated in all cortical progenitors from E10.5, we showed that the hippocampus is reduced and displaced, and the dentate gyrus is particularly affected in adult mutant mice. In particular, the temporal progression of granule cell migration is perturbed. With the help of a second conditional mutant line in which COUP-TF1 is inactivated solely in post-mitotic neurons but maintained in progenitor cells, we confirmed that COUP-TF1 functions specifically in mitotic compartment. Our results indicate that COUP-TF1 is implicated in regulating particular aspects of stem cell development and migration, and propose COUP-TF1 as a novel factor required in hippocampus development.

Oral Communications - Session 4/OC n°2

# The pathological state before the onset of Epilepsy

*Jennifer Lavigne*, Renaud Bussière, Camille Liautard, Marion Ayrault, Massimo Mantegazza*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Epilepsies are chronic, often severe, neurologic disorders. In order to develop effective and targeted therapies, it is essential to clarify differential pathomechanisms of different epilepsies. Sodium channels are key players of neuronal excitability and are important targets of epileptogenic mutations. Hundreds of mutations have been identified in the SCN1A gene (the Nav1.1 sodium channel α-subunit); they can cause a large spectrum of epileptic phenotypes but here, we have focused on two genetic epilepsies due to the mutation of this gene and exhibiting variable phenotypes: Generalized Epilepsy with Febrile Seizures plus (GEFS+) and Dravet Syndrome (DS). In these pathologies, there is a latency period after birth during which changes are taking place in neuronal networks leading to chronic epilepsy, but the precise mechanisms generating seizures and different phenotypes are largely unknown. Methods: we have studies dysfunctions of hippocampo-entorhino-cortical and thalamo-cortical networks in the two murine models of genetic epilepsies that carry the Nav1.1 mutations in pré-epileptic periods, investigating intrinsic excitability of neurons with patch-clamp, network disruptions with field potential recording and studies in vivo on seizures, monitored with an electrocorticogramme (ECoG). Results: We observed a hypoexcitability of inhibitory neurons without changes in excitatory neurons and traces of hyperexcitability in the neuronal networks of young animals before onset of first seizures. We can induce seizures using increase of body temperature after P21 (corresponding to chronic epilepsy) in order to test the effect of different treatments administered for preventive during pre-epileptic periods.

Oral Communications - Session 4/OC n°3

# Alpha-linolenic acid supplementation impoves motor and cognitive functions in a mice model of stroke

*M. Bourourou*, O. Le Thuc, C. Rovère, C. Heurteaux, and N. Blondeau*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

**Introduction:** An emerging direction to combat stroke that is one of the leading causes of death and disability worldwide is nutraceuticals. This term aims promoting the concept of foodstuffs as therapeutics. In this context, we have previously demonstrated in a mice model of ischemic stroke that the omega-3 polyunsaturated fatty acid, α-linolenic acid (ALA) triggers neuronal protection and neuroplasticity. ALA supplementated daily diet also prevents mortality and cerebral damage. We thus hypothesized that ALA supplementation might facilitate recovery of motor and cognitive function post-stroke.

**Materials and Methods:** ALA supplementation was achieved by an experimental diet enriched in ALA by a factor of three compared to regular chows. The ALA enriched diet did not contain any EPA and DHA, while the regular chow did, in proportions already matching the "murine" recommended intake. After a 6-week diet, stroke was induced by introducing a filament into the middle cerebral artery (MCA) of anesthetized mice. Infarct was assessed on Cresyl violet stained sections 24h-post-stroke. Motor deficits were assessed in the rotarod and pole tests and cognitive deficits in the Morris water maze test.

**Results:** The ALA supplemented mice did not display a reduced the lesion, while, they showed an increased latency to fall off the rotarod from the day 2 to day 4 after stroke and reduced latency to reach the floor at day 3. ALA supplementation decreases the time to find the platform during the training days and the time to enter the platform location on the test day during the second week of recovery post-stroke.

**Conclusion:** Our preclinical research highlights the interest of ALA nutritional supplementation to reduce post-stroke mortality, neuronal damage and promote rehabilitation.

Oral Communications - Session 4/OC n°4

# Childhood Onset Schizophrenia: Epidemiology, Clinical and Neurocognitive Exploration, Relationship With the Autism Spectrum Disorders

*E. Dor*, B. Bardoni, F. Askenazy*

**Cognition Behavior Technology (CoBTek) Laboratory, Université Nice Sophia Antipolis, Nice*

Background: Childhood-Onset Schizophrenia (COS) is a rare but an extremely serious psychiatric pathology presenting an alteration of the basic functions. The child will introduce major distortions of sensory perceptions, thinking, emotions and motor behavior. This creates important individual, family and societal consequences. Described in 2 forms: Very Early Onset Schizophrenia (VEOS), beginning before age 12 and Early Onset Schizophrenia (EOS), beginning between 12 to 18 years. COS remains a poorly understood clinical entity because of its low incidence (1 0/0 to 30/0) and the difficulty of the clinical diagnosis. Moreover, links between the Autism Spectrum Disorders (ASD) and the VEOS are not yet well known. Neuro-developmental pathologies share clinical similarities and differences that are investigated by the international community with the purpose to determine their clinical, neurocognitive, neurobiological and genetic specificities. Methods: According to our clinical experience and literature data, we hypothesize that, COS is not diagnosed with standardized clinical assessments among the population of children cared in long-term structures in France. Then, our first work is an epidemiological study whose main objective is to assess the prevalence of the COS in medical and health institutions in 3 sub regions of the PACA. Then, we have the opportunity to characterize the clinical and neurocognitive COS cohort and to assess the number of children with a dual diagnosis of COS and TSA. This sub - group of children appears to be a strong genetic salience phenotype. So we started a clinical and genetic family study which aims is: to characterize the clinical and neurocognitive profile of whole family and to identify mutations in the subgroup of patients by the sequencing of the genome with the strategy of the trios. Results: 29/302 children have a COS diagnosis (9.7%), 7/29 have TSA diagnosis. 9 families, 30 subjects included, genome study ongoing for 4 trios.

# Dissecting the pro-tumoral role of the CD98/LAT1 complex

*Y. Cormerais*, J. Durivault, S. Giulano, R. LeFloch, B. Front, S.K. Parks, L. R de la Ballina, M. Palacin, N.M. Mazure and J. Pouysségur*

**Centre Scientifique de Monaco (CSM), Département de Biologie Médicale, Monaco*

The CD98/LAT1 heterodimer is a multifunctional, HIF induced, transmembrane complex that is overexpressed in many cancers and described as a bad prognostic marker. The CD98 glycoprotein of the CD98/LAT1 heterodimer interacts with integrins to regulate migration and adhesion-induced intracellular signaling. The LAT1 component of the complex mediates the transport of essential large neutral amino acids in mammals. Previous studies by Féral et al. (PNAS, 2005), have shown that CD98 knock-out mice display restricted teratocarcinoma formation. Despite the fact that CD98 knock-out mice have reduced activity of LAT1, this pro-tumoral action was reported to be rather due to the key role of integrins via CD98 signaling than to the transporter LAT1 itself. We challenged this hypothesis by predicting that LAT1 is the most pro-tumoral component. In this talk we will discuss the pro-tumoral role of each protein using genetic ablation of LAT1 or CD98 in cancer cells.

# The SYK tyrosine kinase negatively regulates β1 integrin function and migration of melanoma cells

*Emilien Garcia*, Mélanie Tichet, Aude Mallaviale, Damien Alcor, Nicholas Dubois, Zied Djabari, Marcel Deckert and Sophie Tartare-Deckert*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

The progression of tumors to the metastatic disease involves the loss of metastatic suppressor functions. The propensity to metastasize is particularly high in melanoma, an aggressive skin cancer. Thus, aberrant cell migration is a key feature of melanoma progression, and is required for metastasis. Consequently, (epi)genetic changes that dysregulate cell motility, are important for melanoma malignancy. SYK (Spleen Tyrosine Kinase) is a cytoplasmic tyrosine kinase that has been implicated in tumor suppression of melanoma. SYK is frequently downregulated by epigenetic silencing and its loss has been associated with senescence escape, but whether it also regulates tumor migration remains poorly understood. In this work we used gain- and loss-of-function approaches to analyze SYK's effects on migration abilities of human and murine melanoma cells. We found that reexpression of SYK or knockdown of SYK results in decreased or increased migration and invasion of melanoma cells, respectively. Notably, SYK knockdown cells displayed a mesenchymal-like phenotype with upregulation of Fibronectin, SPARC and TWIST1. In vivo, reexpression of SYK in SYK-deficient cells decreased their ability for lung colonization. Interestingly, a kinase deficient mutant showed the same ability of wild-type SYK to suppress migration and metastasis, indicating that this effect is likely independent of the enzymatic activity. Mechanistically, we found that silencing of SYK upregulated integrin β1 activity that resulted in increased focal adhesion numbers, FAK-dependent signaling and enhanced melanoma cell adhesion. Interfering with β1 integrin function using blocking antibody prevented enhanced adhesion elicited by SYK knockdown. Our study unveils a novel role for SYK in suppressing integrin-mediated adhesion, a process that functions both as points of traction and as signaling platform during cell migration and outlines the importance of SYK inactivation in acquisition of metastatic potential.

Oral Communications - Session 5/OC n°3

# Implication of E2F1 transcription factor in melanoma

*Florian Rouaud*, Patricia Abbe, Michaël Cerezo, Abdelali Lehraiki, Marina Boncompagnie, Magali Plaisant, Emilie Jaune, Robert Ballotti and Stéphane Rocchi.*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

Melanoma is a very aggressive tumor for which there is currently no efficient treatment. E2F transcription factors family are known to regulate the expression of genes involved in cell cycle. Aberrant expression of E2F1 transcription factor has been found in high-grade tumors associated with poor prognosis including metastatic melanoma. Although E2F1 overexpression is found in many cancers, its role remains controversial. Considering data of the literature, our hypothesis is that blocking the E2F1 pathway might have a therapeutic benefit for the treatment of melanoma. In this study, we showed that E2F1 is overexpressed in several melanoma cell lines and in melanoma cells freshly isolated from patients. We also found that E2F1 inhibition by RNA interference or a pharmacological inhibitor, leads to cell death through apoptosis, senescence characterized by a typical morphology, and biochemical changes associated with cell cycle arrest in G2/M. Cell death, senescence and cell cycle arrest induced by the inhibition of E2F1 were shown to be dependant of p53 and p27 pathways resulting in the generation of ROS and DNA damage. Moreover, we showed that p53 mutated cells are resistant to the induction of apoptosis, senescence and cell cycle arrest induced by E2F1 inhibition. These data reinforce our hypothesis concerning the involvement of p53 in E2F1 effects, suggesting a potential role of E2F1 in the treatment of melanoma.

# Role of *Xeroderma pigmentosum* fibroblasts in squamous cell carcinoma cell invasion

*S. Al-Qaraghuli* and T. Magnaldo*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

Squamous cell carcinoma (SCC) is the most frequent metastatic skin cancer. The etiology of skin SCC is linked to exposure to genetic stress, notably ultraviolet radiation (UVR). *Xeroderma pigmentosum* type C (XP-C) is a rare genetic disorder characterized by a severe susceptibility to particularly aggressive SCCs following minimal exposure to UVR, hence compromising the life expectancy of patients. XP-C cells are deficient in the nucleotide excision repair mechanism (NER) of DNA lesions introduced at bipyrimidine sequences upon UVR exposure. XP-C dermal fibroblasts constitutively expressed a phenotype resembling that of stromal fibroblasts associated to cancer cells (CAFs). XP-C fibroblasts accumulate ROS and MMP1. We further explored the phenotype of XP-C fibroblasts and found that they constitutively overexpress hepatocyte growth factor/scatter factor (HGF/SF) leading to activation of the HGF receptor cMET, and hence P38 activation. In organotypic skin cultures, XP-C fibroblasts promoted the invasion of SCC cells. Scratch-Healing of SCC cells was enhanced in the presence of culture supernatants of XP-C fibroblasts. XP-C fibroblasts acted on SCC cells through a mitogenic effect. Specifically, ratio of SCC cells in the G2-M phase of the cell cycle was augmented by 50 % in the presence of XP-C fibroblasts culture supernatants. Conversely, blockage of c-MET activation led to prevented invasiveness of SCC cells within dermal equivalent, most probably through the inhibition of p38 activation. Spheroid assays showed that XP-C fibroblasts led the collective invasion of SCC cells. Together, our data indicated for the first time that absence of XPC in fibroblasts led to overexpression of HGF and other factors such as MMP1 promoting SCC cells proliferation and invasion. Pharmacological approaches capable of neutralizing the proinvasive and mitogenic effect of XP-C fibroblasts may be considered as treatments in XP patients as well as skin cancer in general populations.

Oral Communications - Session 5/OC n°5

# Study of PINK1 in Human Tumor Development

*T. Goiran*, E. Duplan, J. Viotti, J. Honorat, C. Alves Da Costa, F. Checler*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Several epidemiological data evidenced a negative correlation between Parkinson disease (PD) and cancer. This negative correlation raises the question of whether despite phenotypically distinct, these two diseases could share common molecular effectors. Corroborating this hypothesis, it is worth noting that most of familial PD-associated proteins are implicated in cell cycle and cell death control and are often abnormally expressed in a varied range of tumor types. Given the privileged crosstalk between several PD gene products and p53, a tumor suppressor strongly implicated in gliomagenesis, we have decided to investigate the interplay between p53 and PINK1. PINK1 is a serine threonine kinase associated to autosomal recessive PD which has been implicated in the control of mitochondrial integrity and function. PINK1 harbor anti-apoptotic and proliferative properties and its expression is affected in several cancer types. We demonstrate for the first time a molecular link between p53 and PINK1 ex-vivo and in-vivo corroborating a role of PINK1 in brain tumors development.

# Regulation of the conversion of white to brown adipocytes by arachidonic acid metabolic pathway

*Rayane A. Ghandour*, Didier F. Pisani, Jean-Claude Chambard, Maude Giroud, Michel Tauc, Alexandros Vegiopoulos, Stephan Herzig, Dominique Langin, Gérard Ailhaud, Christophe Duranton and Ez-Zoubir Amri*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

The recent discovery of functional brown adipocytes in adult humans has led to the consideration of their use to increase energy expenditure in the treatment of obesity and associated diseases. We aimed to study the effect of an excess of poly-unsaturated fatty acids ω6 on brown adipocytes formation. We used a unique cell model for studying human brown adipocyte formation, the hMADS cells (human-Multipotent Adipose-Derived-Stem). We analysed the effect of arachidonic acid (ARA) or its metabolites, prostaglandins (PGs) of series 2, on the formation of hMADS brown adipocytes. Our data showed different effects of the prostaglandins studied. PGF2α and PGE2 inhibited the conversion of white into brown adipocytes through a pathway involving intracellular calcium, MAPK and PPARγ. Meanwhile, PGE 2 was also able to induce this conversion via a cAMP-dependent pathway. This dual capacity might be due to the diversity of EP membrane receptors and their different signaling pathways. Finally prostacyclin (PGI2) induced this conversion through a pathway involving PPARs and IP receptor. These results showed that the effect of ARA on the conversion of white into brown adipocytes depends on three factors: i) the nature of prostaglandins synthesized ii) the secreted amount and iii) the presence of different receptors on adipocytes membrane. Our results suggested that in addition to promoting the formation of white adipocytes, excess of polyunsaturated fatty acids in diets might increase their deleterious effect by altering the process of "browning" in the white adipose tissue.

# Pancreatic somatostatin-expressing cells: An untapped source for beta-cell regeneration?

*N. Druelle*, A. Shabro, M. Courtney, B. Hadzic, S. Navarro and P. Collombat*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Affecting an expanding number of people, Type 1 Diabetes Mellitus is an autoimmune disorder which results in the loss of insulin-producing beta-cells in the islet of Langerhans. Therefore, approaches aiming at gaining further insight into the molecular mechanisms underlying beta-cell (neo)genesis, during pancreas morphogenesis and throughout adulthood, is of growing interest. Toward this goal, a network involving numerous transcription factors was found to progressively specify endodermal progenitor cells toward the pancreatic, endocrine, and ultimately islet cell fates. Among these, Arx and Pax4, were found to exert key roles for the allocation to the alpha-/PP- and beta-/delta-cell fates, respectively. Importantly, we recently showed that adult alpha-cells can be regenerated and converted into functional beta-like cells upon the sole ectopic expression of Pax4. Surprisingly, an increase in the number of somatostatin-expressing delta-cells was also noted in these animals, such cells not accumulating over time. One could therefore wonder whether delta-cells could also be regenerated and converted into beta-like cells. To this end, using two different transgenic mouse lines, delta-cell plasticity was investigated by focusing on two hypotheses: (1) Can somatostatin-expressing cells be converted into beta-like cells following Pax4 misexpression in alpha-cells? (2) Do somatostatin+ cells have the ability to convert into beta-like cells upon Pax4 misexpression? Combining lineage tracing experiments and immunofluorescence, we show that Pax4 misexpression in alpha-cells results in the neogenesis and conversion of delta-cells into beta-like cells. Furthermore, our latest results also suggest that the sole ectopic expressing of Pax4 directly in delta-cells is sufficient to induce a massive insulin-producing cell hyperplasia. Our current work will be presented.

# Regulation of Adipogenic Lineage in Healthy and Dystrophic Human Muscles

*Claudine Moratal*, Nicole Arrighi, Christian Dani, Claude A Dechesne*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Human skeletal muscle is a powerful regenerative tissue in response to a variety of injuries. The muscle regeneration can be deficient in dystrophic muscles such as in patients affected by Duchenne muscular dystrophy (DMD). This neuromuscular disease is characterized by infiltrations of fibrotic and adipose tissues which limit the success of therapies. Intramuscular fibro-adipogenic progenitors (FAPs) could be at the origin of these intramuscular fibrotic and adipose deposits in DMD patients since their number is higher in DMD muscles than in healthy muscles, and they can differentiate into white adipocytes and myofibrolasts. The aim of my thesis is to assess the hypothesis that FAPs regulation is disturbed in DMD muscles compared to healthy muscles resulting to their differentiation into adipocytes and myofibroblasts in dystrophic patients. Myogenic progenitors (MPs) are main actors of muscle regeneration forming new myofibers to substitute damaged myofibers, and could be a potential regulator of FAPs. Healthy FAPs/MPs co-cultures demonstrated cell interactions between these two intramuscular progenitors implicating soluble factors. Indeed, FAPs adipogenesis is highly decreased whereas FAPs fibrogenesis is increased. In addition, MPs myogenesis is significantly increased in co-cultures. On the other hand, MPs stimulate FAPs proliferation, and MPs proliferation is decelerated by FAPs. The molecular mechanisms of these cell interactions involve the signaling pathways TGFβ and Hedgehog. Interestingly in DMD, MPs did not regulate FAPs although FAPs modulated MPs proliferation and differentiation, as observed for healthy progenitors. Together, these results could explain infiltrations of adipose and fibrotic tissues in skeletal muscles of DMD patients. In the long term, these data could be important in the discovery of new targets for the development of a treatment to increase efficiency of current therapies.

Oral Communications - Session 6/OC n°4

# Role of primary cilium during adipocyte differentiation

*Nicolas Forcioli-Conti*, Sandra Lacas-Gervais, David Estève, Anne Bouloumié, Christian Dani, Pascal Peraldi*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

The Primary cilium is an organelle present in most of the cells of the organism. Ciliopathies are genetic disorders of the primary cilium and can be associated with obesity. We have studied the primary cilium during adipocyte differentiation of human adipose stem cells (hASC). We have observed that the size of the primary cilium follows several modifications during adipocyte differentiation. It is absent in growing cells and appears in confluent cells. Interestingly, during the first days of differentiation, the cilium undergoes a dramatic elongation that can be mimicked by dexamethasone alone. Thereafter, its size decreases. It can still be detected in cells that begin to accumulate lipids but is absent in cells that are filled with lipids. Moreover, the cilium elongation does not seem to affect the localization of proteins associated with the cilium such as Kif3-A or Smoothened. However, Hedgehog signaling, an anti-adipogenic pathway dependent on the primary cilium is inhibited after three days of differentiation, concomitantly with the cilium size increase. On the other hand, we observed that the levels of acetylated α-tubulin, a major constituent of the primary cilium, and the expression of HDAC6, the enzyme that deacetylates α-tubulin and is responsible for the loss of the cilium during mitosis, are modulated during adipogenesis. We observed that inhibition of HDAC6 activity leads to a decrease in adipocyte differentiation. This is associated with an inhibition of the initial elongation of the cilium. Interestingly, over expression of HDAC6 inhibits adipocyte differentiation and blunts the elongation of the primary cilium. This indicates that HDAC6 controls adipogenesis through the levels of acetylated α-tubulin. Moreover, we show that although HDAC6 expression increases during adipocyte differentiation it is not sufficient to provoke the loss of the cilium. This suggests the existence of another mechanism for the loss of the cilium.

Oral Communications - Session 6/OC n°5

# A new role for Monocyte chemoattractant protein 1/CCL2 in promoting weight loss through inhibition of melanin-concentrating hormone-expressing neurons.

*Ophélia Le Thuc*, Nicolas Blondeau, Alice Guyon, Julie Cazareth, William Rostène, Catherine Heurteaux, Jean-Louis Nahon and Carole Rovère.*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Injuries or infections induce endocrine, autonomic and behavioural changes known as "sickness behaviour". Among them, fever and weight loss appear driven by hypothalamic cytokines, although the exact mechanism remains elusive. Our goal was to investigate the neuroimmunological events driving appetite and weight loss in systemic high-grade inflammation. To study the role of the hypothalamic inflammation in the inflammation-driven weight loss, we performed intracerebroventricular (ICV) injections of lipopolysaccharide (LPS) in C57Bl6/J male mice. Blood and cerebral tissues were collected: mRNA and protein levels of cytokines/chemokines and hypothalamic peptides involved in the regulation of food intake were measured. The effect of inflammatory factors on neuropeptidergic systems involved in food intake was investigated via experiments of hypothalamic neuropeptides release (perifusion) and by electrophysiology. A central injection of LPS provokes a temporal sequence linking activation of pro-inflammatory cytokines and chemokines (notably CCL2) to down-regulation of the orexigenic neuropeptide Melanin-Concentrating Hormone (MCH). CCL2 particular activation kinetics lead us to investigate whether CCL2 could mediate LPS effects. ICV-injected CCL2 triggers neuroinflammation, downregulation of MCH and weight loss. Furthermore, CCL2 reduces KCl-induced MCH release from perifused hypothalamic explants and hyperpolarizes MCH neurons. These effects are reversed by the CCR2 antagonist INCB 3344 and in CCR2-deficient mice. Finally, the demonstration that MCH neurons expressed CCL2 receptor confirms that CCL2 could act directly on MCH-neurons promoting inflammation associated weight loss. In conclusion, CCL2 appears as a major intermediate between cytokine-producing cells and neurons in the cascade linking inflammation and eating disorders as LPS-induced weight loss is mediated by CCL2 up-regulation through modulation of the MCH neuronal network.

# Targeting miR-125b-5p to fight obesity via browning of white adipose tissue

*M. Giroud*, V. Barquissau, M. Karbiener, D. Pisani, A. Mairal, G. Beranger, D. Beuzelin, R. Ghandour, JC. Chambard, T. Balaguer, M. Scheideler, D. Langin and EZ. Amri*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

The recent discovery of functional brown adipocytes in adult humans has led to the consideration of their use to increase energy expenditure in the treatment of obesity. Furthermore, in rodents and humans, islands of brown adipocytes, termed "brite" (brown-in-white) adipocytes, emerge within white adipose tissue (WAT) after cold exposure or β3-adrenergic receptor stimulation. The identification of factors which trigger an increased mass/activity of human brite adipocyte is of great interest for the treatment of obesity. One of these factors is microRNA known to interfere with the translation of messengers in proteins. Using hMADS cells, able to differentiate into human white adipocytes and convert into functional brite adipocytes, we identified miR-125b as down regulated upon this conversion. In human and rodent, miR-125b expression was down regulated in BAT compared to WAT. miR-125b mimic transfection in hMADS brite adipocytes decreased basal oxygen consumption and maximal mitochondrial respiration. We showed that miR-125b levels were down regulated in sub-cutaneous (sc) WAT and in BAT upon both in vivo β3-adrenergic receptor stimulation and cold exposure, and thus associated with BAT activation and brite recruitment. Finally, we found that miR-125b mimic injection in scWAT inhibited β3-adrenergically-induced UCP1 expression and mitochondriogenesis. Our observations are in favor of an important role of miR-125b in the control of white to brite adipocyte conversion via regulation of mitochondriogenesis. miR-125b loss of function should allow a better understanding of the mechanisms underlying the role of this miRNA in the browning of white adipocytes, paving a way for the development of new therapies.

Oral Communications - Session 7/OC n°1

# The study of Dilp8, a new hormone coordinating organ growth

*Emilie Boone*, Julien Colombani, Ditte Andersen*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Growth of different body parts needs to be coordinated and scaled with the overall body size to give rise to adults of correct proportions. Since different organs follow autonomous growth programs and therefore grow at different speeds and during distinct stages of development, mechanisms must operate to ensure that each organ has reached an appropriate size before proceeding through developmental transitions. We recently identified Drosophila insulin-like peptide 8 in a genetic screen for molecules coupling organ growth with developmental transitions. Dilp8 is secreted from abnormally growing tissues and acts on the brain complex to delay pupariation. dilp8 expression levels drops at the end of larval development suggesting a coupling between autonomous organ growth programs and dilp8 expression. Identifying signals that regulate dilp8 expression during development is therefore likely to provide an understanding of organ size assessment mechanisms. The Hippo tumour suppressor pathway plays a major function in restricting organ growth by promoting cell cycle exit and apoptosis. Hippo signalling is highly responsive to the mechanical forces operating in growing organs. Activation of the Hippo pathway restricts nuclear translocation of the transcriptional co-activator Yorkie, which together with its DNA-binding partner Scalloped, regulates downstream growth-promoting target genes. We show here that Yorkie is necessary for inducing dilp8 expression and the associated delay in pupariation. Using a molecular biology approach, we demonstrate that Scalloped/Yorkie binds directly to three Hippo Responsive Elements (HREs) located in the dilp8 promoter. A minimum promoter encompassing the three HREs is sufficient to activate dilp8 transcription in vitro and in vivo. We propose that dilp8 is a direct target of the Hippo pathway and its expression levels inversely correlates with organ size allowing a coupling between autonomous organ growth programs and animal maturation.

# WT1 controls antagonistic FGF and BMP-pSMAD pathways in early renal progenitors

*Fariba Jian Motamedi*, Danielle A. Badro, Michael Clarkson, M. Rita Lecca, Stephen T. Bradford, Fabian A. Buske, Kathrin Saar, Norbert Hu¨bner, Andre W. Brandli & Andreas Schedle*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Kidney organogenesis requires the tight control of proliferation, differentiation, and apoptosis of renal progenitor cells. The transcriptional regulator WT1 is required for renal progenitor survival, but the molecular mechanism underlying its function in this cell population remains elusive. Here we used ChIP-Seq combined with microarray analysis to identify a set of renal progenitor specific genes that depends on Wt1 gene function. We demonstrate that lack of Wt1 abolished FGF and induced BMP signaling within the metanephric mesenchyme. Apoptosis associated with loss of Wt1 could berescued by addition of recombinant FGFs or by inhibiting BMP/pSMAD signaling. Wefurther show that recombinant BMP4, but not BMP7, induces an apoptotic response within the early kidney that can be suppressed by simultaneous stimulation with FGF. Thus FGF and pSMAD pathways act in an opposing manner within the early metanephric mesenchyme with WT1 holding a key role by directly regulating FGF signaling pathway genes.

# A PLA2R1 epitope study in Membranous Nephropathy: diagnostic and prognostic significance

*Guillaume Dolla**

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

The phospholipase A2 receptor (PLA2R1) is the major autoantigen in idiopathic membranous nephropathy, with two recently identified epitopes of unknown clinical significance. Here, fifty PLA2R1-positive patients' sera were screened by western blot on a series of PLA2R1 deletion mutants covering the ten extracellular domains. We identified epitopes in several distinct domains and confirmed the reactivity against these domains with soluble forms of each domain. Domain-specific ELISAs allowed stratifying 69 PLA2R1- positive patients into subgroups depending on the epitopes their sera react to. Each groups had significantly distinct severity outcome. We conclude that analysis of the PLA2R1 epitope profile during follow-up is a powerful tool to monitor disease severity and stratify patients into subgroups with different renal prognosis.

# The cortical patterning gene COUP-TFI regulates major components of neuronal activity during development

*E. Magrinelli*, C. Alfano, K. Harb, M. Mantegazza, M. Studer*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Neocortical arealization is a developmental process in which a set of patterning genes shapes the primordial pallial neuroepithelium into a structure subdivided into several tangential domains with distinct functions. Several lines of evidence suggest that spontaneous neuronal activity may also modulate transcriptional regulators and neuronal circuits in the developing cerebral cortex. However, it is still unclear whether and how neuronal activity acts on the areal organization of the neocortex during development and at early postnatal stages. We demonstrate that COUP-TFI, major areal patterning gene, promotes cortical sensory identity and connectivity primordially in post-mitotic cells. Hence it is plausible that COUP-TFI shapes cortical maturation modulating expression of genes involved in neuronal activity, as already described for the olfactory bulb. However, it is still unclear how the expression of patterning genes influences the functional properties of the neocortical neurons during development. Our data indicate a direct and indirect involvement of COUP-TFI in the transcriptional regulation of a set of activity-related genes. We used genetic loss- and gain-of-function approaches that allowed us to identify a consistent number of candidate genes involved in activity and acting downstream of COUP-TFI from early to late post-natal ages. We are now testing whether and how these candidates participate in the areal and laminar defects previously described in COUP-TFI mutant cortices. This study aims to unraveling a possible link between areal-specific organization of cortical circuitry and neuronal activity by taking COUP-TFI as a model system. Understanding the mechanisms that allow the neocortex to differentiate from an immature state to a functional mature one, not only increases our comprehension on this complex phenomenon, but also contributes in understanding the aetiology of severe neurodevelopmental disorders in which neuronal activity is altered.

Oral Communications - Session 7/OC n°5

# Human induced pluripotent stem cells from Andersen's syndrome patients: Implication of the Kir2.1 potassium channel in bone morphogenesis

*Jonathan Pini*, Matthieu Rouleau, Sabrina Sacconi, Claude Desnuelle, and Saïd Bendahhou*

**Laboratoire de PhysioMédecine Moléculaire (LP2M), UMR CNRS 7370 - Université de Nice Sophia Antipolis, Nice*

Andersen's syndrome (AS) is a rare disorder characterized by a triad of symptoms: periodic paralysis, cardiac arrhythmia and bone developmental defects. Most of the patients carry mutations on the inward rectifier potassium channel Kir2.1 encoded by the KCNJ2 gene. Kcnj2 knockout mice are lethal at birth preventing hence thorough investigations of the physiological and pathophysiological events leading to muscle dysfunction and bone malformation. We have generated induced pluripotent stem (iPS) cells from healthy as well as from Andersen's syndrome patient muscular biopsies by infection of myoblasts with lentivirus, containing the 4 genes required for cellular reprogramming (Oct4, Sox2, Klf4 and cMyc). The generated AS-iPS cells expressed genetics and surface pluripotent markers and could be differentiated, by formation of embryoid bodies, into the three germ layers. To go further, we differentiated patient and control iPS cells into mesenchymal stromal cells (MSC). Cells obtained from both iPS cell types exhibited the same characteristics: expression of MSC markers and lack of expression of hematopoietic markers. Patient and control MSCs differentiated into adipocytes. Control MSC could differentiate into osteoblasts but patient MSCs had a lower osteoblast differentiation ability. Control MSC that were treated with low BaCl2 concentrations, a specific inhibitor of the Kir2.1 channel, had a low ability to differentiate into osteoblasts than untreated cells. Our results show that functional Kir2.1 channels are not important for the reprogramming process; the early step of the development in vitro and for the differentiation into mesodermal cells such as MSC, and adipocytes. However, the lower ability of cells, lacking the kir2.1 channels, to differentiate into osteoblasts shows that functional Kir2.1 channels are crucial to the osteoblastogenesis.

# POSTERS

## *Abstracts*

Poster n°1

# Effect of lipids on Acid Sensing Ion Channel 3 and pain

*S. Marra*, A. Delaunay, M. Christin, V. Friend, S. Sebille, C. Cognard, J. Noël, E. Lingueglia, E. Deval*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Acid-sensing ion channel 3 (ASIC3) is an excitatory ion channel activated by extracellular acidification. It is expressed in peripheral sensory neurons, where it has been involved in the detection of inflammatory and post-operative pain (Deval et al, 2011 ; Deval et al, 2008 ; Ikeuchi et al, 2008 ; Sluka et al, 2007). In this study, we show that ASIC3 is not only able to sense changes in extracellular pH, but is also activated by endogenous lipids (arachidonic acid and lysophospholipids) produced following hydrolysis of the plasma membrane by phospholipase A2. Activation of ASIC3 by lipids is reversible and occurs at physiological pH7.4, i.e., without any acidification of the extracellular medium. The effect of lipids is progressive, leads to a constitutive depolarizing current which is blocked by the ASIC3-inhibitory peptide APETx2, and is most probably due a direct interaction with the channel. Moreover, lipids are able to activate native ASIC3 currents in DRG neurons and induce an increase in nociceptive C-fibers firing. Finally, lipids also induce a pain behavior in rats which is partially inhibited by pharmacological blockers of ASIC3 channels. Lysophospholipids and arachidonic acid are thus endogenous signals able to trigger activation of ASIC3 in the absence of extracellular acidification. These findings further confirm the important role of ASIC3 as a sensor of inflammatory mediators at the peripheral level, and it opens new perspectives about the role of this channel in lipid signaling.

Poster n°2

# The small G-proteins Arf2 and Arl1 are critical for *Candida albicans* filamentous growth and virulence

*H. Labbaoui*, S. Bogliolo, S. Filler, R.A. Arkowitz & M. Bassilana*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

The switch from budding to filamentous growth, critical for the virulence of the human fungal pathogen *Candida albicans*, requires sustained polarized growth, as well as reorganization of cellular compartments and continued exo- and endocytosis. In the budding yeast *Saccharomyces cerevisiae*, Arf G-proteins and their regulators have been shown to function at the interface of membrane traffic and cell polarity, raising the question of their role in *C. albicans* external-signal mediated filamentous growth. *C. albicans* has 3 Arf proteins, Arf1-Arf3 and 2 Arf-like proteins, Arl1 and Arl3. Our results indicate that only Arf2 is required for viability in this organism. This is in contrast to the situation in *S. cerevisiae*, in which Arf1 or Arf2 alone are not required for viability, but together are essential. We examined the importance of these Arf/Arl proteins in *C. albicans* filamentous growth, cell wall integrity, antifungal sensitivity and virulence. In contrast to Arf1, Arf2 mutants are defective in cell morphology, filamentous growth, cell wall integrity, as well as being hypersensitive to antifungal drugs. Arl1 is required for invasive filamentous growth and cell wall integrity, but not antifungal sensitivity. In addition, the hyphae in the *arl1* mutant were 2-fold shorter compared to the WT. Whether the hyphal elongation rate or hyphal initiation is altered in this mutant is under investigation. The *arl3* mutant had an intermediate phenotype between the WT and the *arl1* strains. Finally, we determined that the *arf2* and *arl1* mutants were substantially reduced in virulence both in hematogenously disseminated and oropharyngeal candidiasis murine models. In contrast, although Arf3, the homolog of human Arf6, is required for polarized growth in *S. cerevisiae*, in *C. albicans*, it is not required for invasive filamentous growth or virulence. All together, our results highlight the importance of Arf2 and Arl1 for the bud to hyphal transition, critical for *C. albicans* virulence.

Poster n°3

# Phospholipase D2 specifically regulates TREK potassium channels via direct interaction and local production of phosphatidic acid

*Y Comoglio*, J Levitz, MA Kienzler, F Lesage, EY Isacoff, G Sandoz.*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Membrane lipids serve as second messengers and docking sites for proteins and play central roles in cell signaling. A major question about lipid signaling is whether diffusible lipids can selectively target specific proteins. One family of lipid-regulated membrane proteins is the TWIK-related K channel (TREK) subfamily of K2P channels: TREK1, TREK2, and TWIK-related arachdonic acid stimulated K(+) channel (TRAAK). We investigated the regulation of TREK channels by phosphatidic acid (PA), which is generated by phospholipase D (PLD) via hydrolysis of phosphatidylcholine. Even though all three of the channels are sensitive to PA, we found that only TREK1 and TREK2 are potentiated by PLD2 and that none of these channels is modulated by PLD1, indicating surprising selectivity. We found that PLD2, but not PLD1, directly binds to the C terminus of TREK1 and TREK2, but not to TRAAK. The results have led to a model for selective lipid regulation by localization of phospholipid enzymes to specific effector proteins. Finally, we show that regulation of TREK channels by PLD2 occurs natively in hippocampal neurons.

Poster n°4

# Tandem pore domain Halothane-Inhibited K+ channel subunits THIK1 and THIK2 assemble and form active channels

*Sandy Blin*, Franck C Chatelain, Sylvain Feliciangeli, Dawon Kang, Florian Lesage and Delphine Bichet*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Two-pore domain potassium channels are involved in several functions or diseases such as neuronal excitability, apoptosis, breathing, epilepsy, cancer, pain … Despite a high level of sequence homology, tandem pore domain halothane-inhibited K+ channel 1 (THIK1) produces background K+ currents whereas THIK2 is silent. This lack of activity is due to a unique combination of intracellular retention and weak basal activity in the plasma membrane. Here, we designed THIK subunits containing dominant-negative mutations (THIK1DN and THIK2DN). THIK2DN mutant inhibits THIK1 currents whereas THIK1DN inhibits an activated form of THIK2 (THIK2-A155P-I158D). In situ proximity ligation assays and FRET experiments support a physical association between THIK1 and THIK2. Next, we expressed covalent tandems of THIK proteins to obtain expression of pure heterodimers. Td-THIK1-THIK2 produces K+ currents of amplitude similar to Td-THIK1-THIK1 but with a noticeable difference in the current kinetics. Unlike Td-THIK2-THIK2 that is mainly detected in the endoplasmic reticulum (ER), Td-THIK1-THIK2 distributes at the plasma membrane indicating that THIK1 can mask the ER retention/retrieval motif of THIK2. Kinetics and unitary conductance of Td-THIK1-THIK2 are intermediate between THIK1 and THIK2. Altogether, these results show that THIK1 and THIK2 form active heteromeric channels, further expanding the known repertoire of K+ channels.

Poster n°5

# Application of a Computational Homology Modeling Approach to Characterize Substrates and Inhibitors of CYP26A1 and CYP26B1

*Robert Foti*, Nina Isoherranen, Philippe Diaz, Dominique Douguet*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Cytochrome P450 26A1 and 26B1 are members of the cytochrome P450 family of enzymes which catalyze the metabolism of all-trans retinoic acid, an endogenous molecule which plays key roles in the homeostasis of multiple cellular mechanisms through interactions with retinoic acid receptors. In the absence of crystal structures for either CYP26A1 or CYP26B1, homology models that provide structural information and are supported by experimental data may prove to be extremely valuable for characterizing substrates and inhibitors of these enzymes. The main aim of this study was to design computational models of CYP26A1 and CYP26B1 in order to characterize ligand binding characteristics which lead to catalysis and/or inhibition of the enzymes. The models were initially validated using all-trans retinoic acid as a ligand and then employed to characterize the binding of tazarotenic acid, an active metabolite formed by the deesterification of tazarotene. All computational results were evaluated in vitro through the use of metabolite identification experiments. Next, as CYP2C8 has also been shown to metabolize all-trans retinoic acid, inhibition of CYP26A1 and CYP26B1 by a set of known CYP2C8 inhibitors was evaluated using tazarotenic acid as an in vitro probe substrate for CYP26A1 and CYP26B1. The binding of select inhibitors in the active sites of CYP26A1 and CYP26B1 was rationalized using the respective homology models. Molecular analysis of the active sites of estimated the active site volumes of CYP26A1 and CYP26B1 to be 918 Å3 and 977 Å3, respectively, suggesting that the enzymes are capable of binding "drug-like" compounds. Ultimately, the evaluation of the active site characteristics and in vitro activity of CYP26A1 and CYP26B1 support an expanded role for the enzymes in the metabolism of xenobiotic compounds.

Poster n°6

# Role of Rab G Proteins in the human fungal pathogen *Candida albicans*

*Rohan Sanjay Wakade**

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

*C. albicans* is a harmless commensal that can become a pathogenic. The dimorphic switch, a key feature critical for its virulence, requires active cytoskeleton reorganization and sustained membrane traffic. Rab G Proteins have important functions in membrane traffic, yet less is known in their role in external signal mediated polarized growth and pathogenicity. *C. albicans* has 8 small Rab G proteins, compared to 11 in *Saccharomyces cerevisiae*. We focused on Rab proteins that are most likely to function in the Golgi, endosomes and plasma membrane i.e. Sec4, Ypt31 and Ypt6. In *S. cerevisiae* Sec4 and Ypt31/32 plays role in exocytosis where as Ypt6 plays a role in endocytosis. In *C. albicans*, Sec4 and its activator Sec2 were found at the Spitzenkorper and Cdc28/ Hgc1-dependent Sec2 phosphorylation was shown to be critical for hyphal growth . We examined the roles of Ypt31 and Ypt6, relative to Sec4, in *C. albicans* filamentous growth and cell wall integrity, together with their importance for antifungal resistance. Our data shows that like Sec4, Ypt31 is essential for viability. Also, Sec4 and Ypt31 are similarly required for filamentous growth, both in liquid and solid inducing conditions, in addition to being critical for cell wall integrity and antifungal sensitivity. Whereas Ypt6 was not essential for viability but required for invasive filamentous growth and cell wall integrity; ypt6 deletion mutant cells formed hyphae with reduced lengths. The Rab and Arf family GTPases play a pivotal role in membrane trafficking and it was shown in *S. cerevisiae*, that Rab and Arl proteins could functionally overlap, as overexpression of ARL1 complemented the defect of a ypt6 mutant. To characterize Arl1 and Ypt6 interactions in *C. albicans*, YPT6 and ARL1 were overexpressed in the wild-type, arl1 ypt6 mutant strain. Our data shows that, overexpression of YPT6 solely complements invasive filamentous defect of an arl1 mutant.

Poster n°7

# Regulation of Cubitus interruptus in the Hedgehog signalosome complex

*Cécile Giordano*, Laurent Ruel, Pascal Thérond*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Hedgehog (Hh) is a morphogene that controls in a gradient-dependent manner cell growth and differentiation in both invertebrates and vertebrates. In Drosophila, the Hh signal transduction is initiated by interaction of Hh with its receptor Patched that induces Smoothened (Smo) activation. This leads to signal transmission to a cytoplasmic complex composed of four proteins: the kinase Fused (Fu), the protein kinase A (PKA), the kinesin like Costal2 and the transcription factor Cubitus interruptus (Ci). But it remains unclear how the Hh signalosome complex regulates the activity of Ci. To answer this question, I analysed, by using Bimolecular Fluorescence Complementation, the conformational changes that occurs in the transduction complex depending on Hh signal in Drosophila. I first demonstrated that the PKA/Ci association takes place within the cytoplasmic complex, and showed the Hh-mediated disruption of PKA from Ci is independent of the catalytic activity of Fu, but dependent on Smo. Secondly, I also showed that Fu interacts on two different domains of Ci depending on Hh signal. In absence of Hh, the complex Fu/PKA interacts with Ci, and this proximity allows to the PKA to phosphorylate Ci leading to the formation of Ci as a transcriptional repressor formation. In presence of Hh, PKA is relocalised from Ci to Smo proteins whereas the protein Fu moves from the C-terminal domain to N-terminal domain of Ci. This process allows to the Fu kinase to regulate Ci as a transcriptional activator, probably by direct phosphorylation. All together my data revealed conformational changes of two different kinases, PKA and Fu within the protein complex involved in Hh transduction. Depending on Hh signal, PKA and Fu associate with Ci in order to generate different isoforms and different activities of Ci. This project will go further by the study of Fu/Ci interaction in vivo, but also by elucidating the relationship between Smo and Fu/Ci which is essential to Ci regulation.

Poster n°8

# Regulation of HACE1 by post-translational modifications

*Maria Isabel Acosta Lopez**

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

Rac1 is a small GTPase involved in regulation of actin cytoskeleton dependent processes, like adhesion, migration, cytokinesis and phagocytosis. It switches between an inactive GDP-bound state and an active GTP-bound state, where it is able to bind to its effectors. As expected for such a central signaling module, Rac1 is tightly regulated within the cell by interaction with activators (GEFS) and inactivators (GAPS). In addition, our team found a new mode of regulation consisting in the ubiquitination of ative Rac1 and its subsequent proteosomal degradation. We also identified HACE1 as the E3 ligase that targets active Rac1. HACE1 has been described as a tumor suppressor, downregulated in several cancer types, and a protective agent against oxidative stress. Furthermore, many works have shown that de-regulation of Rac1 ubiquitination either through Rac1 mutations or HACE1 inhibition are correlated to rac1-driven cancer progession. Despite this, little is known about HACE1 regulation or signaling context. Therefore, the objective of my thesis is to identify how HACE1 is regulated by post-translational modifications, specifically by phosphorylation. We have identified by mass spectrometry one phospho-site in HACE1 that is modulated by Rho GTPases activation. This site inhibits the ability of HACE1 to ubiquitilate Rac1, indicating a positive feedback regulatory module. It seems that the inhibition of HACE1 by phosphorylation does not act through a loss of enzyme-substrate affinity. Ongoing work is aimed to pinpoint how phosphoryation modulates HACE1 activity and which kinases are responsible for modifying HACE1. So far this work describes a new way of regulation for HACE1 and implies a more dynamic regulation of Rac1 by HACE1.

Poster n°9

# Lipid and G-protein interactions in *C. albicans* responding to chemical and physical inducers of filamentous growth

*Patricia Silva*, Martine Bassilana, Robert Arkowitz*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

The localization of phospholipids and activated small GTPases coincides with polarized growth and movement in a range of cell types, suggesting that the specific distribution of these molecules may be important for such processes. *Candida albicans* is a harmless commensal fungus, which in immuno-compromised individuals can cause superficial as well as life-threatening systemic infections. *C. albicans*'s success as a pathogen results from its ability to switch between a budding form and a filamentous hyphal form. Phosphatidylinositol phosphates and activated Rho GTPases are critical for *C. albicans* morphogenesis (1-3). To determine whether the specific localization of these lipids and activated GTPases is important for morphogenesis we are targeting these factors to the plasma membrane (PM) and investigating whether this is sufficient to *i*) compete with existing growth sites or *ii*) to prevent these growth sites from initiating filamentous growth, in a wild type; *iii*) to generate new sites of growth in a mutant defective for filamentous growth. To specifically recruit small GTPase activators and phosphatidylinositol phosphate kinases to the PM, we are using the blue light-activated CRY2/CIBN system from *A. thaliana*. We have implemented this system with one domain constitutively targeted to the PM and the other in the cytoplasm. Photo-activation substantially increases the affinity of the cytoplasmic domain for the other domain, resulting in its recruitment to the PM. We are currently optimizing the CRY2/CIBN system in *C. albicans* and determining if we can recruit different PIP kinases and small GTPase activators to the PM. Full length kinases/GTPase activators or defined catalytic domains are being tested to determine if they can recover the function of the respective mutants. We are also switching the location of the CRY2 and CIBN domains, to enable site specific PM recruitment and ultimately investigate its effect on filamentous growth.

Poster n°10

# Characterization of long non-coding RNAs modulated by hypoxia in early stage lung adenocarcinomas

*Laura Moreno-Leon*, Marius Illie, Richard Allan, Nicolas Nottet, Agnès Paquet, Nathalie Mazure, Nicolas Pottier, Pascal Barbry, Paul Hofman, Bernard Mari and Roger Rezzonico*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Non Small Cell Lung Cancer (NSCLC) 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. While it is now clear that the non coding genome is functionally important, its role in cancer development is largely unexplored. Considerable attention has notably focused on "long non-coding RNAs" (lncRNAs) which control gene expression through various mechanisms involving the recruitment of protein partners. Recent findings indicate that their expression profiles are deregulated in various cancers and that some lncRNAs can be regulated by hypoxia or inflammatory processes. A profiling study on 50 pairs of early-stage lung adenocarcinomas (ADC) samples from the Nice Tumor Biobank indicated that a specific lncRNA signature was associated with a metagene hypoxic signature, previously associated with the aggressiveness of early-stage NSCLC. Interestingly, some of these transcripts were also sensitive to hypoxia in the A549 ADC cell line. We focused on 4 "hypoxaLinc" (H3, H4, H6 and H7) that were significantly up-regulated both in tumors and in various ADC cell lines in response to hypoxia. Notably, we characterized LincH3 and showed that this 10-11kb nuclear transcript was composed of 6 exons and could modulate gene expression through transcription regulation or epigenetic modification of chromatin organization. Its induction by hypoxia was dependent on NFκB activation, and accordingly, LincH3 was also stimulated by the proinflammatory cytokine, IL-1β. Currently, we are analyzing the impact of LincH3 knockdown using gapmer oligonucleotides on the transcriptome of ADC cells under hypoxic conditions. We propose that a better understanding of the role and mode of action of these hypoxaLinc may represent a significant advance for the management of lung cancer patients.

Poster n°11

# Genomic influence on integration site selection by human L1 retrotransposons

*Tania Sultana*, Oliver Siol, Dominic Van Essen, Claude Philippe, Simona Saccani, Nicolas Gilbert, Gaël Cristofari*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

Retrotransposons are mobile genetic elements that employ an RNA intermediate and a reverse transcription step for their replication. Long Interspersed Nuclear Elements-1 (LINE-1 or L1) form the only autonomously active retrotransposon family in humans. Although most copies are defective due to the accumulation of mutations or deletions, each individual genome contains an average of 100 retrotransposition-competent L1 copies, which contribute to the dynamics of our contemporary genome. The core retrotransposition machinery is a ribonucleoprotein particle (RNP) containing the L1 mRNA, and with endonuclease and reverse transcriptase activities. It initiates reverse transcription directly at genomic target sites upon endonuclease cleavage, a process known as target-primed reverse transcription (TPRT). The sequence specificity of the endonuclease, as well as base-pairing between the L1 mRNA and the target site, contributes to L1 target choice. However, whether L1 exhibits a preference for specific genomic locations beyond small sequence determinants is currently unknown. To address this question, we induce retrotransposition by transfecting a plasmid-borne L1 element into human cell lines. This copy contains an artificial sequence tag, which allows us to discriminate novel L1 insertions from existing ones. The chosen cell lines have been extensively studied in the context of the ENCODE project. Hence large ChIP-seq data for various histone modifications or transcription factor binding sites are publicly available. De novo integration events are then mapped by deep-sequencing using a dedicated method developed in the laboratory (ATLAS-seq). Finally, new integration sites will be compared to genomic features (such as gene body, introns, exons, promoters, histone marks, transcription factor binding sites, trnascription start sites, replication origins, DNAse sensitivity, etc). This study will highlight how the genomic context influences L1 target site preference in vivo.

Poster n°12

# Gene expression control by novel classes of small non-coding RNAs in lipid-laden macrophages

*Z. Hizir*, E. Repetto, M. Trabucchi*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

Small RNAs play a role in many biological processes. Recently, my team and others have used high-throughput transcriptomic analyses, documenting the importance of the generation of novel classes of small. Recently, we have identified a new regulatory class of small-RNAs, referred to as s-RNYs. s-RNYs originate from the processing of RNYs and form a ribonucleoprotein complex with Ro60. Using macrophages treated with pro-atherogenic factors, we have found that s-RNYs induce apoptosis and inflammation. Investigating the mechanisms of Ro60/s-RNY complex in regulating cell death and the inflammation in lipid-laden macrophages, we hypothesize that Ro60/s-RNYs complex modulates gene expression at post-transcriptional level. To validate this hypothesis I am working to determine the direct target(s) RNAs of s-RNYs and the binding features that characterize s-RNY target regulation in macrophages by HITS-CLIP analysis. I foresee that understanding the molecular mechanism(s) underlying the regulation of Ro60/s-RNYs expression has significant biomedical implications. Indeed, because s-RNYs are consistently involved in the inflammatory response mediated by macrophages, and are deregulated in atherosclerosis-related diseases, we predict that developing strategies to modulate their expression and function would have a beneficial effect on several groups of patients.

Poster n°13

# Potential therapeutic, diagnostic and prognosis roles of miR-199a-5p in Idiopathic Pulmonary Fibrosis

*Matthieu Buscot*, Gregoire Savary, Nihal Martis, Bernard Mari*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Idiopathic Pulmonary Fibrosis (IPF) is a fibroproliferative disease of unknown origin responsible for a progressive and irreversible loss of lung function. Despite recent advances in clinical management and therapeutic approaches the survival remains poor with a median of 3.5 years after diagnosis. The formal diagnosis is made on an open lung biopsy which is rarely possible in these respiratory compromised patients. The course of the disease is unpredictable with highly heterogeneous phenotypes ranging from slow progression to fulminating respiratory failure. There is thus a need for alternative therapeutic approaches and novel biomarkers discovery for diagnosis and prognosis. Our team has previously identified and characterized a microRNA, miR-199a-5p, involved in the myofibroblast maintenance and activation via the facilitation of the TGF-b pathway. The most relevant identified target of the miR is caveoline-1 (CAV-1), which is known to be an anti-fibrotic regulator. This mechanism represents a potential and original therapeutic target. Two inhibition strategies have been considered: (1) direct inhibition of miR-199a-5p by an antisense oligonucleotide, (2) CAV-1 protection by preventing the binding of miR-199a-5p to the specific 3'UTR domain. We have managed to demonstrate the anti-fibrotic effect of an anti-miR-199a-5p in a murine bleomycin-induced lung fibrosis model. The second inhibition strategy is still being assessed. Serums at diagnose from patients with IPF have been collected. Each donor has been perfectly phenotyped, particularly concerning the speed of function decline. Healthy donors plasma have been collected as well. Small RNA sequencing will be used to assess the differential expression profiles of circulating miRs. miRs or combinations of miRs with diagnosis or prognosis values will be identified. All together, there results should improve the scientific knowledge and the clinical management of patients with this devastating disease.

Poster n°14

# Characterization of root-knot nematode effectors targeting host nuclear functions

*N.M Truong*, C.-N. Nguyen, J. Mejias, L. Perfus-Barbeoch, M. Magliano, M. Da Rocha, E.D.J. Danchin, P. Abad, B. Favery and M. Quentin*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Plant parasitic nematodes are microscopic worms, the most damaging species of which have adopted a sedentary lifestyle within their hosts. These obligate endoparasites are biotrophs that induce the differentiation of root cells into hypertrophied, multinucleate feeding cells. Effectors synthesized in the esophageal glands of the nematode are injected into the plant cells via the syringe-like stylet and may be required to modulate many aspects of plant cell morphogenesis and physiology leading to the establishment of the feeding giant cells. In a search for *Meloidogyne incognita* effectors targeting to the giant cell nuclei, we used bioinformatics and comparative genomics on EST and NGS datasets to identify genes encoding proteins potentially secreted upon the early steps of infection. We identified genes specifically expressed in the esophageal glands of parasitic juveniles that encode predicted secreted proteins and have a Nuclear Localization Signal and/or a DNA-Binding Domain. In planta nuclear localization of these putative effectors was confirmed using tobacco agro-infiltration, and siRNA soaking was used to silence these genes and study their role during parasitism. Using a yeast-two-hybrid approach and BiFC, we aim at identifying host nuclear functions manipulated by these effectors.

Poster n°15

# A Secreted MIF Cytokine Enables Aphid Feeding and Represses Plant Immune Responses

*Elodie Naessens*, Géraldine Dubreuil, Philippe Giordanengo, Olga Baron, Naïma Minet-Kebdani, Harald Keller and Christine Coustau*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Aphids attack virtually all plant species and cause serious crop damages in agriculture. Despite their dramatic impact on food production, little is known about the molecular processes that allow aphids to exploit their host plants. To date, few aphid salivary proteins have been identified that are essential for aphid feeding, and their nature and function remain largely unknown. Here, we show that a macrophage migration inhibitory factor (MIF) is secreted in aphid saliva. In vertebrates, MIFs are important pro-inflammatory cytokines regulating immune responses. MIF proteins are also secreted by parasites of vertebrates, including nematodes, ticks, and protozoa, and participate in the modulation of host immune responses. The finding that a plant parasite secretes a MIF protein prompted us to question the role of the cytokine in the plant-aphid interaction. We show here that expression of MIF genes is crucial for aphid survival, fecundity, and feeding on a host plant. The ectopic expression of aphid MIFs in leaf tissues inhibits major plant immune responses, such as the expression of defense-related genes, callose deposition, and hypersensitive cell death. Functional complementation analyses in vivo allowed demonstrating that MIF1 is the member of the MIF protein family that allows aphids to exploit their host plants. To our knowledge, this is the first report of a cytokine that is secreted by a parasite to modulate plant immune responses. Our findings suggest a so-far-unsuspected conservation of infection strategies among parasites of animal and plant species.

Poster n°16

# Identification of epigenetic marks in the plant parasitic root-knot nematode *Meloidogyne incognita*

*Loris Pratx*, Laetitia Perfus-Barbeoch, Philippe Castagnone-Sereno, Céline Cosseau, Cristoph Grunau, Pierre Abad*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Root-knot nematodes, such as *Meloidogyne incognita*, are obligatory plant parasites that constitute major agricultural pests worldwide. Our knowledge about *M. incognita*'s genetics regulation has significantly increased since genome sequencing, transcriptomic analysis and gene annotations are now available. However, despite this knowledge, the "classical" genetics fails to understand some phenomena occurring in our model. *M. incognita* reproduces in an asexual way by parthenogenesis without meiosis. Genetically identical individuals develop from females and form clonal populations. Although these clones share the same genetic heritage, modifications of their phenotype can be observed when they are exposed to unfavorable environments. For instance, the virulence (i.e. capacity to parasite a resistant crop) is heritable but transmitted in a non-Mendelian way (not acquired by 100% of the "clonal daughters") and could not be associated to a modification in DNA sequence. Epigenetic modifications can drive phenotypes by other mechanisms than genetics. These modifications are heritable, but metastable, which could change phenotypes by modifying genomic expression. We propose to test role of epigenome in the generation of phenotypic variability and consequently for microevolution towards infection success. We detailed DNA methylation and nucleosome structure, carriers of epigenetic information. We also developed a ChIP-seq assay protocol to compare post-transcriptional histone modifications between virulent and avirulent parasites ; and between different developmental stages. Our preliminary data indicated that the genome of *M. incognita* is not methylated and confirmed the existence of histone modifications which represents important markers involved in gene activation or repression by modifying chromatin state.

Poster n°17

# Comprehensive Transcriptome Profiling of Root-knot Nematodes During Plant Infection and Characterization of Species-Specific Traits

*C.-N. Nguyen*, L. Perfus-Barbeoch, M. Quentin, E.G.J. Danchin, M. Da Rocha, M. Magliano, P. Abad and B. Favery*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Root-knot nematodes (RKN) are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks. They infect roots as microscopic vermiform second-stage juveniles (J2) and migrate between cells to reach the plant vascular cylinder. To further develop and molt into a pear-shaped female that will release hundreds of eggs on the root surface, J2s need to successfully establish and maintain specialized feeding structures called "giant-cells" from which they withdraw water and nutrients. Our project aims to identify RKN genes specifically involved in plant parasitism with an emphasis on genes encoding new secreted effectors. Using Illumina RNA-seq technologies, we compared transcriptomes of Meloidogyne incognita during its life cycle and identified genes over-expressed in early parasitic stages as compared to pre-parasitic juveniles (J2s), eggs, females and males. Once the over-expression of selected genes in parasitic stages was confirmed by RT-qPCR, in situ hybridizations were carried out to localize the candidates in the nematode secretion organs. Furthermore, siRNA soaking was used to silence these genes and study their role in pathogenicity. In parallel, we are also comparing the transcriptomes of M. incognita with those of another RKN species that reproduces by obligatory parthenogenesis, M. enterolobii. This nematode represents a new threat for the agriculture worldwide because of its ability to reproduce on the majority of known RKN-resistant plants. This comparison will allow us to identify, not only the common set of effectors, but also those specific to one of the other RKN species and possibly involved in host range differences.

Poster n°18

# Characterization of small regulatory RNAs involved in the establishment of giant cells induced by parasitic nematodes of genus *Meloidogyne*

*C. Medina*, M. Magliano, M. Da Rocha, B. Favery, P. Abad and S. Jaubert-Possamai*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Plant response to bioagressors involves modifications of gene expression. Recently, microRNAs have been evidenced as crucial regulators of host gene expression during plants-pathogen interactions. Root-knot nematodes (RKN) are biotrophic plant parasitic worms that transform plant cells from root vascular cylinder into hypertrophied, multinucleate and highly metabolically active giant feeding cells. Since RKN are able to induce the formation of feeding cells in roots of almost all cultivated plants, they are thought to manipulate essential and conserved plant molecular pathways. Previous transcriptomic analyses evidenced that redifferentiation of root cells into giant feeding cells implies transcriptional reprogramming with a large repression of gene expression. Our study aims to investigate the role of microRNAs in the regulation of transcriptional repression observed during the redifferentiation into feeding cells. Small RNAs from *Arabidopsis thaliana* roots infected with the RKN model species *Meloidogyne incognita* were sequenced by SOLID technology. As a control, small RNAs from non infected roots were also sequenced. First, a catalog of microRNA expressed in healthy and infected roots was established. Then, microRNAs that are differentially expressed between healthy and infected roots were then identified by Deseq statistical analyses. Preliminary results show that only few microRNAs are differentially expressed in infected roots and statistically relevant. Interestingly some of these microRNAs are evolutionary conserved in plants and are known as main factors of plant response to biotic and abiotic stresses or of interaction with symbiotic bacteria. Our results suggest that microRNAs are involved in the regulation of gene expression that results in redifferentiation of root cells into giant feeding cells. Moreover some microRNA-networks appear to be shared by plant response to biotic and abiotic stress.

Poster n°19

# Production of biological control agents: does genetics matter? The cases of hybridization and inbreeding

*Bastien Quaglietti*, Xavier Fauvergue, Thibaut Malausa*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Classical and augmentation biological control consist in field release of beneficial organisms (the biological control agents - BCA) for crop protection against harmful organisms. These methods rely on the mass-production of BCA. Maintaining both BCA quality and mass-production efficiency is a key challenge. Mass-produced populations may come from hybridization between several natural populations, may have been founded from a few individuals, may undergo repeated demographic bottlenecks during their rearing and are also supposed to evolve under selection to industrial conditions. These mechanisms may impact positively or negatively both BCA quality and production efficiency. Despite its importance in biological control, short-term evolution of reared populations has very rarely been experimentally investigated. The aim of my PhD is to apply fundamental knowledge in evolutionary biology to provide experimental results allowing the improvement of BCA mass-production. My work focuses on 2 axes: first, the exploitation of genetic variability to improve quality and yield of production through hybridization and creation of new strains. Second, the detection and assessment of inbreeding and inbreeding depression which could decrease individual fitness and production yields. My PhD is integrated in a large project financed by the European Commission (FP7-IAPP "Colbics") focusing on public/private collaborations in biological control. Experimental approaches are carried out in 3 organizations from public (INRA, France) and private (Biobest, Belgium and Anasac, Chile) sectors, all facing BCA production challenges. So far, 4 species have been used: 2 predatory insects (*Macrolophus pygmaeus* & *Chrysoperla carnea*), 1 parasitoid wasp (*Allotropa burrelli*) and 1 predatory mite (*Neoseiulus cucumeris*). The results obtained so far pave the way for a set of methodological developments in mass-production for several of these insects.

Poster n°20

# Physiological and biochemical responses to water deficit in Faba bean (*Vicia faba*), inoculated with Haouz native rhizobia

*Ablaa Kabbadj*, Bouchra Makoudi, Mohammed Mouradi, Nicolas Pauly, Pierre Frendo and Cherki Ghoulam*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice* and *Unit of Plant Biotechnology and Symbiosis Agro physiology, Faculty of Sciences and Techniques, Marrakech*

Because of aridity and the high pressure of increasing human population, water became a scary expensive commodity, especially in developing countries affected by drought. This later abiotic stress causes a reduction of crop productions especially in the more sensitive cultures. Through their ability to fix atmospheric nitrogen via symbiosis with soil rhizobia, legume species are considered as an interesting integral component of sustainable agricultural production systems. Faba bean (*Vicia faba L.*) is one of the most cultivated grain legumes in Morocco. Its production is constrained by abiotic factors such as drought. Thus, the use of drought tolerant cultivars could be a promising way to improve faba bean production in affected areas. This study aimed to evaluate the physiological and biochemical responses of two faba bean varieties to water deficiency (Fb1& Fb2) inoculated with native rhizobia strains (Rs1& Rs2) isolated from Haouz region. Four symbiotic combinations were studied and compared under two soil moisture levels (80 % and 40 % of field capacity). The results showed that water deficit induced decrease of shoot and nodule dry weight and this reduction was more pronounced in Fb2 inoculated with Rs1 and Rs2. Relative water content (RWC) and chlorophyll content decreased significantly under stress for both Fb2/Rs1 and Fb2/Rs2. Moreover, biochemical response to water deficit revealed that Fb2/Rs1 presented the highest increase of malonyldialdehyde (MDA) content in leaves and nodules. This change was accompanied by an important increase of hydrogen peroxide (H2O2) content. A lower accumulation of MDA and H2O2 was obtained in Fb1/Rs1. Furthermore, peroxidase and catalase activities were higher for this couple. Otherwise, Fb1/Rs1 was more able to maintain the H2O2 content by the mean of increased antioxidant enzyme activities.

Poster n°21

# Is the futur of The Antarctic fur seal (*Arctocephallus gazella*) getting darker?

*Lauriane Massardier-Galatà*, Jennifer Morinay, Fredéric Bailleul, Eric Wajnberg, Christophe Guinet, Patrick Coquillard*

**Institut Sophia Agrobiotech (ISA), UMR INRA 1355 - CNRS 7254 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

A noticeable southward shift of the highly productive frontal systems serving as the main foraging areas for many top predator species is likely to occur over time in response to climate warming in the Antarctic and Subantarctic areas. Seabirds and pinnipeds are thus likely to be faced with an increase in the distance between foraging locations and their land-based breeding colonies. The Antarctic fur seal is widely distributed across the southern oceans and extensive bio-energetic data are available for this species. During the pup-rearing period, females commute between the land, where they suckle their offspring, and the sea, where they feed. A spatially explicit individual-based model, including bio-energetic components, was built to evaluate the effects of possible changes in the abundance, aggregation levels, and distance of prey resources on pup growth and survival of both female and pup in relation with females' body size and behavioural traits. The aggregation of resources and the body size of the seal had major effects. When we controlled for prey abundance and distance from the colony, females were found to be most successful for levels of moderate prey aggregation, resulting in optimal body size with respect to environmental conditions. As a consequence, phenotypic traits, such as female body length, should vary in the population as a function of prey distribution. We therefore predicted that an increase in the distance separating the prey from the colony will drive the selection of longer individuals. Moreover we attempt to verify the existence of such optimal values of the maternal body-size, related to environmental parameters of the simulations, as suggested by the results. Since this distance is supposed to depend on climate parameters (particularly the average temperature), we aim at estimating the potential impact of an increase of the distance on the population dynamics with several scenarios of climatic changes.

Poster n°22

# ARIH1, a novel regulator of mitophagy in cancer cells

*Elodie Villa*, Emma Proïcs, Camila Rubio-Patiño, Sandra Lacas-Gervais, Sandrine Obba, Barbara Zunino, Ophélie Meynet, Sandrine Marchetti and Jean-Ehrland Ricci*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

One particularity that cancer cells develop to counteract cell death is to selectively eliminate impaired mitochondria. Damaged mitochondria can be removed by a process called mitophagy. The main actor of this process is the E3 ligase PARKIN. It is mainly overexpressed in brain and when mutated (in Parkinson's disease), the mitophagy is defective. PARKIN belongs to the RBR (RING-in-between-RING) family that is composed of 11 other members differentially expressed depending on the tissues. PARKIN is the only E3 ligase known to induce mitophagy of the damaged mitochondria. However knowing that i) mitophagy is central to remove damage mitochondria from cancer cells and ii) PARKIN is mainly expressed in the brain, we wondered if other RBR E3 ligases could participate in the mitophagy of damaged mitochondria in cancer cells? We established that ARIH1, which is found overexpressed in several cancer lines, participate to cancer maintenance by removing damaged mitochondria. Indeed, when we treated cells overexpressing ARIH1 with a mitochondrial uncoupling agent (CCCP), we observed a decrease in the mitochondrial mass indicating the occurrence of mitophagy. We also established that this ARIH1–dependent mitophagy was dependent on the presence of PINK1, a kinase required to recruit ARIH1 (or PARKIN) to the damaged mitochondria. In conclusion, our work provides a new insight in the regulation of the mitophagy process and identifies ARIH1 as a new molecular actor of this pathway in cancer cells.

Poster n°23

# CD98hc is required to maintain the functional and dynamic interface between the epidermis and the underlying dermis

*Floriane Tissot*, Laurence Cailleteau, Etienne Boulter, Lionel Tosello, Soline Estrach, and Chloé C. Féral*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

The interaction between epidermis and dermis is crucial for many processes from skin development to homeostasis and tumor formation. While this crosstalk involves integrins, its regulations are poorly understood. Here, we investigated the role of the transmembrane protein CD98hc, an integrin co-receptor and amino acid transport chaperon, in skin compartment crosstalk. To do so, we generated CD98hc deficient mice specifically in dermal fibroblasts using compound mice in which CD98hc is floxed and the Cre recombinase, under Fsp1 promoter, is constitutively expressed. Interestingly, beyond obvious dermal phenotypes, we observed epidermal defects during skin homeostasis. Dermal loss of CD98hc delays hair growth and impairs wound healing. Moreover, we show that dermal CD98hc is required for proper basal keratinocyte layer organization during homeostasis and proliferation in a mechanically induced disruption of the epidermal barrier model. To get further insights into the underlying mechanisms, we used an in vitro keratinocytes and fibroblasts coculture assay. We demonstrate that fibroblast feeder layer expression of CD98hc is necessary for efficient colony forming efficiency (CFE) of WT primary keratinocyte populations. Importantly, the few keratinocyte clones formed on a CD98hc deficient feeder layer were all abortive, suggesting a strong proliferation defect. Interestingly, CFE was improved with partial rescue of keratinocyte proliferative capacity, by adding WT feeder conditioned media onto the co-culture. This suggests the possible role of diffusible factor(s), which we now ought to identify, in CD98hc-mediated skin crosstalk. Altogether, our data point out a novel role for the amino acid transporter and integrin regulator, CD98hc, in providing skin with an efficient and dynamic epidermis/dermis interface.

Poster n°24

# Carcinoma Associated Fibroblasts-dependent matrix remodeling RNAi screening identified ICAM1 as a regulator of pro-invasive tumor microenvironment

*S. Bonan*, J. Albrengues, I. Bourget, C. Gaggioli*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

Carcinoma associated fibroblasts (CAF) are key components of solid tumor ecosystems. CAF are responsible for remodeling of the extracellular matrix (ECM), thereby they contribute to generation of tensile forces in the tumor microenvironment that promote favorable cues for tumor development and metastasis. We previously demonstrated that the pro-inflammatory cytokine LIF (Leukemia Inhibitory Factor) initiates the pro-invasive stromal fibroblast phenotype in cancers. Similarly to CAF, LIF-activated human primary dermal fibroblasts (hPDF) sustain a constitutive pro-invasive phenotype. Today, how the permanently activated status of CAF is sustained remains unclear. Gene expression profile analysis of LIF-activated hPDF identified a subset of 64 significantly-modulated genes both after 48h-hours and 15 days of stimulation by LIF. Among these, based on their functions, we selected 50 genes of which we studied the possible role in activating the fibroblasts' capacity to promote matrix remodeling. Using collagen and laminine-rich matrix contraction assays, small interference RNAs specific to the candidate gene were used to assess their ability to counteract the activity in both human CAF and LIF-activated fibroblasts. Nine genes were thus selected. Among them, ICAM1 (Intercellular Adhesion Molecule 1), a transmembrane protein member of the immunoglobulin superfamily involved in cell-cell communication, was identified as the most potent regulator of CAF contractility in our in vitro system. We next demonstrate that inhibition of ICAM1 expression using siRNA or inhibition of ICAM1 activity using specific blocking antibody in CAF block pro-invasive ECM remodeling. Moreover, overexpression of a GFP fused ICAM1 protein in hPDF is sufficient to promote the pro-invasive phenotype of fibroblast. Therefore, we are now investigating the molecular and cellular mechanisms regulated by ICAM1 that mediate the evolution of the fibroblast-dependent pro-invasive tumor ecosystem.

Poster n°25

# Neutrophils confer aggressive features to lung tumor cells via miR-223 transfer

*Joséphine Zangari*, Marius Ilie, Laurie Signetti, Dana Goldoni, Valérie Vouret-Craviari, Baharia Mograbi, Paul Hofman and Patrick Brest*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

When interacting with tumor cells, immune cells induced responses that include cell death, resistance and dissemination. In particular, increase in intra-tumoral neutrophils has been shown to be associated with poor prognosis, ie relapse and metastasis. However, the precise roles of neutrophils during the process of migration and dissemination are unknown. Interestingly, overexpression of miR-223, a "neutrophil-specific" microRNA, in lung carcinomas is associated with poor metastasis-free survival. The objective of this study is to investigate the consequences of exosome shuttling or membrane exchange between neutrophils and cancer during carcinogenesis. Firstly, we showed that miR-223 is transferred via cell contacts or exosomes to lung cancer cells. Secondly, we analyzed by time course and dose response the specific effect of these interaction and miRNA exchange. Thirdly, we showed that miR-223 post-transcriptionally down-regulates expression of FOXO1 transcription factor by directly targeting its 3-untranslated regions. These factors are subsequently involved in diverse biological processes such as cellular proliferation, stress resistance and apoptosis. In conclusion, we showed that the possible exosome shuttling between neutrophils and cancer cells promote a clear gain of function of cancer cells by triggering cell migration, invasion and chemo-resistance. These findings uncover a new regulatory model, called specific miRNA delivery from neutrophils, which should be exploited for further immune-targeted strategies in cancer.

Poster n°26

# 3D-organotypic invasion assays for chemical inhibitor library screening indentify Cav1 calcium channel blockers as inhibitors of SCC12 collective invasion

*E Grasset*, M Maiel, S Bonan, C Duranton, G Meneguzzi G and C Gaggioli*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

Carcinoma cell collective invasion is defined by migration of clusters of cohesive cancer cells into the peritumoral extracellular matrix (ECM) of cancers from different origins (melanoma, sarcoma, carcinoma from breast, lung, skin). The cellular mechanisms of this cell migration pattern have been recently described; however, the underlying signaling pathways remain poorly understood. Identification of small molecule inhibitors blocking carcinoma collective invasion would potentially benefit patients suffering from aggressive carcinoma. To identify compounds that could hamper tumor cell invasion, we screened small molecule libraries for their capacity to block epidermoïd carcinoma SCC12 cells invasion in vitro using 'three-dimensional" organotypic invasion assays that mimic mesenchymal and epidermal tumor cell interactions in culture. To this purpose, SCC12 cells are cultured on top of a dense collagen and laminin-rich matrix containing carcinoma associated fibroblast (CAF) cells. This model enables SCC12 to invade the ECM in response to CAF activity that remodels the ECM by creating tracks that SCC12 can use collectively to penetrate the matrix. Screening of 378 compounds that target nuclear receptors, kinases, phosphatases, epigenetic factors, ion channel ligands and wnt signaling, identified 52 chemicals that efficiently block collective SCC12 invasion in vitro. Despite the pleotropic panel of inhibitor targets, we specifically selected Cav1 calcium channel inhibitors for further investigations. Indeed, 9 out of 11 Cav1 calcium channel blockers inhibited SCC12 invasion without any detectable toxic effect on SCC12 and CAF cells. Moreover, knockdown of Cav1.1 in SCC12 cells is sufficient to blocks their collective invasion. In light of our results, we can anticipate that Cav1.1 are key regulators of SCC12 collective invasion. The molecular and cellular mechanisms involved in this process are currently under investigation.

Poster n°27

# Pro-tumorigenic role of HACE1 in melanoma

*Najla El Hachem**, Tanesha Naiken, Nadia Habel, Maryline Allegra, Gregory Salez, Yann Cheli, Corine Bertolotto and Robert Ballotti

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

RAC1 is the third most common somatic mutation in melanoma and is considered as an oncogene in this neoplasm. RAC1 functions in several key cellular processes, including cell migration, cell proliferation and survival. The activity of RAC1, small GTPase, is mainly controlled by GAPS, GEFs and E3 ligases. HACE1, a HECT-domain containing E3-Ubiquitin ligase that interacts preferentially with GTP-bound RAC1, catalyzes its polyubiquitylation and promotes its degradation. Therefore, by favoring the degradation of RAC1, HACE1 is considered to act as a tumor suppressor. The role of HACE1 in melanoma has not been studied so far. In multiple melanoma cell lines and in primary melanoma cells, RNAi-mediated depletion of HACE1 does not affect cell proliferation, but inhibits cell migration. Overexpression of HACE1 promotes cell migration of melanoma cells. It seems that HACE1 behave as an oncogene in melanoma cells. We studied the effect of HACE1 inhibition on the main signaling pathways. HACE1-silencing decreases FAK and AKT phosphorylation that might explain the observed inhibition of migration. Co-immunoprecipitation assays allowed us to identify β1 integrin as a new HACE1 interactor, providing thereby a direct molecular link between HACE1 and melanoma cell migration. Together, our results provide new insights into melanoma cell migration and establish a new potential role of the HACE1 in this process. Our work supports a critical tumorigenic role for HACE1 in melanoma progression.

Poster n°28

# In vitro and in vivo analysis of the oncogenic role of Otx2 in medulloblastoma

*Almahdi Chakroun*, Thomas Lamonerie and Nathalie Billon*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Medulloblastoma (MB) is a malignant and invasive tumor of the cerebellum. It comprises four distinct molecular variants: Wnt and Sonic hedgehog (Shh) groups, in which Wnt and Shh signalization are deregulated, respectively, Group 3 (also known as Myc-medulloblastoma) characterized, among other genomic deregulations, by the overexpression and/or amplification of the Myc gene and of the transcription factor Orthodenticle homeobox 2 (Otx2) gene, and Group 4, which is not well characterized. Recently, Kawauchi et al. generated an animal model of Myc-MB using orthotopic transplantation of granule cell precursors (GCPs) overexpressing Myc and a dominant negative form of the tumor suppressor protein P53 into the cerebellar cortex of the mouse. Otx2 is overexpressed in 74% of medulloblastomas and it is also frequently amplified in type 3 MB. During cerebellum development, Otx2 is expressed by GCPs, which have a high proliferation rate. Deregulation of GCPs proliferation may favor oncogenic processes, as seems to occur in the Shh group of MB. We are investigating the role of Otx2 in the control of GCPs proliferation and differentiation using a mouse genetic model where Otx2 is co-expressed with the green fluorescent protein GFP, allowing identification and purification of Otx2-expressing (Otx2+) GCPs from developing cerebellum. Purified cell populations are subjected to different tests of proliferation (Ki67 expression, Edu incorporation) to assess the proliferation rate of Otx2+ versus Otx2- GCPs. Preliminary results show that Otx2+ GCPs have an increased proliferation rate compared to Otx2- GCPs, suggesting that Otx2 may have an oncogenic role in the establishment of MB through the regulation of GCPs proliferation. We are now studying the effect of Otx2 overexpression or silencing on the proliferation rate of Otx2+ GCPs in in vitro studies. Finally, we will test the oncogenic potential of Otx2 in Myc-medulloblastoma using the transplantation model described above.

Poster n°29

# Extracellular matrix produced by BRAFi-resistant melanoma cells promotes phenotypic switching and therapeutic resistance

*Rania Ben Jouira*, Christophe Girard, Ilona Berestjuk, Aude Mallaviale, Charlotte Pandiani, Damien Alcor, Sophie Tartare-Deckert and Marcel Deckert*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

Cutaneous melanoma remains one of the most challenging and difficult cancer to treat because of its high plasticity, metastatic potential and resistance to treatment. New therapies targeting oncogenic BRAFV600E mutation have shown remarkable clinical efficacy. However, drug resistance invariably develops. Thus, the need for improving existing therapies remains critical. Recent studies have indicated that tumor resistance arises from (epi)genetic cancer cell alterations and the tumor microenvironment in which the extracellular matrix (ECM) is a determinant factor. Both stromal and tumor cells contribute to ECM deposition and remodelling during disease progression. Here, we found that BRAF inhibitors (BRAFi)-resistant melanoma cells, but not BRAFi-sensitive cells, abundantly produced matrix proteins and remodelled a pathological 3D ECM displaying fibronectin (FN) and collagen fibers. The deposition of tumor-derived ECM by BRAFi-resistant cells correlated with an exacerbated invasive mesenchymal phenotype characterized by a decreased expression of MITF and increased expression of the epithelial-to-mesenchymal (EMT) transcription factor SLUG. The importance of tumor-derived ECM is underlined by the observation that migration and invasion of resistant cells is impaired by FN knock-down. In addition, BRAFi-resistant cells displayed enhanced beta 1 integrin/FAK and RHO GTPase signaling, two pathways involved in mechanotransduction. As a consequence, BRAFi-resistant cells exhibited increased cellular and biochemical responses to matrix stiffness. Finally, we found that the 3D ECM produced by resistant cells is able to prevent the antiproliferative effect of the BRAFi Vemurafenib on therapy-sensitive melanoma cells. These findings suggest that resistance to targeted therapy is associated with the production by tumor cells of a pathological matrisome consisting of ECM proteins and associated factors that may affect tumor progression and therapeutic response.

Poster n°30

# Implication of REDD1 (Regulated in Development and DNA Damage response-1) in the activation of the inflammasome

*Faustine Pastor*, Karine Dumas, Franck Ceppo, Mansour Djedaini, Mireille Cormont, Frédéric Bost, Jean-François Tanti, Sophie Giorgetti-Peraldi*

**Centre Méditerranéen de Médecine Moléculaire (C3M), Inserm U 1065, Université Nice Sophia Antipolis, Nice*

Obesity associated with insulin resistance is characterized by a chronic inflammation of the adipose tissue associated with an increase in the production of pro-inflammatory cytokines and macrophages infiltration. In this study, we determine the role of REDD1, a negative regulator of mTORC1, in the development of the inflammation of the adipose tissue. REDD1 expression is increased in the adipose tissue of mice subjected to a high fat diet. Since obesity is associated with an increase of circulating lipopolysaccharide (LPS), we have investigated the effect of LPS on REDD1 expression. Injection of LPS in mice increases the expression of REDD1 and inflammatory markers such as IL-1β, TNFα and IL6 in the adipose tissue. In REDD1-KO mice, absence of REDD1 prevents the expression of IL-1β, TNFα and IL6 in response to LPS. LPS combined with ATP activates the inflammasome detected by NLRP3 expression and the cleavage of caspase-1 in macrophages. This effect seems to be dependent of REDD1, since absence of REDD1 prevents the activation of inflammasome in response to LPS. These results suggest that REDD1 participate to the activation of inflammation in macrophages in response to LPS. To elucidate the effect of downregulation of REDD1 expression on insulin signaling pathway, we have used coculture between adipocytes and macrophages to recapitulate the development of insulin resistance in adipocytes. Absence of REDD1 in macrophages restores the insulin signaling pathway. All together, our results suggest that REDD1 participates to the activation of inflammation in macrophages, crucial to promote insulin resistance in adipocytes.

Poster n°31

# Taste of Ten Drugs Frequently Prescribed in Nursing Homes

*J. Lamure*, P. Brocker, S.M. Schneider, R. Collomp, F. Bertin-Hugault*

**Laboratoire Microbiologie Orale, Immunothérapie et Santé (Micoralis), EA 7354, Faculté d'odontologie, Université de Nice Sophia Antipolis, Nice*

Many frail elderly patients are polymedicated. Whether they suffer from dysphagia or cognitive troubles, they are often given blended food, with drugs crushed and mixed into the food. Health Authorities recommend to crush and to administrate crushed drugs separately, for pharmacologic reasons, but the drugs are usually mixed together to facilitate ease of case by nursing staff. Crushed drugs can have a bad taste, leading to drug / food refusal, worsening malnutrition, but this qualitative aspect has been scarcely studied in geriatric populations. The present study aimed to evaluate the taste of the ten drugs most frequently prescribed in nursing homes, in order to determine which drugs are acceptable or not when crushed and mixed into food. Methods: This one step observational study was designed like a food tasting. A jury of healthy 16 volunteers was. Every tablet or capsule was mixed into 100 mL of berry-flavored jelly or apple sauce. It was a blind tasting of 24 verrines, containing the ten drugs randomly distributed, a control without drug and a combination of the 6 top-list drugs. Twelve jelly verrines were followed by 12 apple sauce verrines. Tasters spat the spoonful content out after they had assessed its taste. Each verrine was scored from 0 (bad taste) to 10 (good). Qualitative comments were also recorded. Results: The lowest scores were attributed to the combination of drugs (1.5 + 1.6; 0 to 5), followed by zopiclone (1.9 + 2.3; 0 to 8), clopidogrel (4.3 + 2.1; 1 to 7) and paracetamol (4.6 + 1.8; 1 to 8). All these drugs had a long-lasting bitterness. Zopiclone mixed and alone was qualified as unbearable and one participant exhibited nausea by taking it. . Drug-free jelly and apple sauce were scored 6.7 + 1.4 (4 to 9) and 7.1 + 1.1 (5-9.5), respectively.

Poster n°32

# TH17 TNFα CD4 T cells induce osteoclasts that participate to hematopoietic stem cells egress implicated in the exacerbation of the inflammatory bowel disease (IBD)

*Agathe Boucoiran*, Nourhène Belaid, Lidia Ibanez, Matthieu Rouleau, Claudine Blin and Abdelilah Wakkach*

**Laboratoire de PhysioMédecine Moléculaire (LP2M), UMR CNRS 7370 - Université de Nice Sophia Antipolis, Nice*

Inflammatory bowel disease is a chronic disease characterized by an inflammation of the gastrointestinal tract. 40% of patient with IBD have an osteopenia/osteoporosis due to an increase of osteoclast activity. Pathogenic T cells are maintained in the bone marrow where they participate to the inflammation secreting cytokines, which increase cell damage and bone loss. In a mouse model of IBD induced by transfer of CD4 T cells in Rag1ko immunodeficient mice, we have recently identified a pathogenic Th17 TNFα CD4+ T cell population that migrates in the bone marrow. These cells produce Rank-L that increase osteoclastogenesis and bone destruction. Hematopoietic stem cells (HSC) are maintained in bone marrow niches composed of mesenchymal stromal cells (MSC). In normal condition, osteoclasts have been described to induce HSC egress from bone marrow and their differentiation into lymphoid and myeloid cells that participate to reconstitution of the immune system. We were then interested to investigate the role of osteoclast activity on the mobilization and egress of HSC from the bone marrow during IBD. We have shown that the mouse model of IBD is associated with an increase of HSC and an accumulation of myeloid cells in the colon. Blockage of osteoclast activity with zoledronic acid (ZA) and calcitonin induce a decrease of the clinical symptoms. This phenotype is associated with a decrease of the HSC number in the bone marrow and a decrease of myeloid cells in the colon. IBD mice have an increase of the proportion of MSC in the bone marrow. Transcriptomic analysis of these cells shows that there is an increase of the expression of the genes involved in HSC mobilization and a decrease in those implicated in their retention during IBD. Treatment with ZA or calcitonin seems to partially reverse effect of IBD on the mesenchymal stromal cells. To conclude, osteoclasts seem to have a central importance in IBD by allowing the mobilization of HSC from the bone marrow.

Poster n°33

# Mice exposed to higher levels of sensory, motor, social and cognitive stimuli show altered survival to viral challenge or endotoxin shock associated to increase type I interferon production

*D. Daoudlarian*, M. Guyot, M. Canali, E. Murris, E. Mougneau, N. Glaichenhaus, P. Blancou*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

Rodents housed in an enriched environment (EE) experience enhanced levels of sensory, motor, social and cognitive stimuli compared to animals housed in a standard environment (SE). Previous studies have shown that enrichment promoted neuronal activation as well as signaling and plasticity throughout various brain regions. We have found that mice house in EE are more resistant to Influenza A virus (IAV) lethal infection. In striking contrast, mice housed in EE are less resistant to endotoxin shock compared to SE-housed mice. Analysis of IAV infected SE and EE mice showed more production of type I interferon and higher viral load in EE mice lungs. Furthermore, mRNA Microarray and ELISA experiments showed that EE peritoneal cavity macrophages stimulated in vitro with the synthetic analog of dsRNA poly(I:C) are producing more type I interferon than SE peritoneal cavity macrophages. These results are suggesting that EE peritoneal cavity macrophages are modified in some way, modifying their ability to produce type I interferon.

Poster n°34

# Interactions between innate immune cells and extracellular matrix proteins in cutaneous carcinoma

*Pierre Bourdely*, Caroline Spenlé, Agata Radwanska, Kelly Nouhen, Franck Bihl, Carmelo Luci, Morgane Roulot, Frédéric Brau, Véronique Braud, Ellen Van Obberghen-Schilling, Gertraud Orend, Fabienne Anjuère*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

The skin and other epithelia are immunocompetent tissues in direct contact with the environment and are therefore repeatedly exposed to mutagen agents and inflammatory molecules that contribute to malignant transformation. Malignant transformation of epithelial tissues is controlled by immune mechanisms in place within the epithelium itself. Indeed, it has been well established that a strong lymphocytic infiltration with cytotoxic T lymphocytes (CTL) is associated with a good clinical outcome. Dendritic cells (DC) are key actors of antitumoral immunity that initiate and regulate the development of T cell responses. The ongoing research of my PhD laboratory aims to dissect the fine tune dialog between the epithelium and the tissue-associated innate immune cells among which DC during skin carcinoma development. It has been already determined that DC functions are altered during skin carcinogenesis. The gene expression profiles of those cells at different stages of carcinogenes! is (inflamed skin, pre-cancerous lesions and tumors) revealed that intratumoral DC overexpressed molecules of the extracellular matrix (ECM), suggesting that DC can contribute to the remodeling of the tumoral ECM and that ECM molecules can modulate DC functional maturation.In that context, the main objective of my PhD project is to study the molecular interactions between DC and tumoral ECM as well as the consequences of these interactions on DC functions. To that purpose, I used two mouse models of skin carcinomas as well as an in vitro model of 3D matrix expressing or not the fibronectin (FN) or the tenascin C (TNC), two major molecules of the ECM.The proposed project will contribute to gain knowledge on the fine-tune dialog between tumoral stroma and DC during skin carcinoma development and will help to develop targeted immunotherapeutic approaches that restore DC antitumoral properties.

Poster n°35

# A link between steroid signaling pathways and L1 retrotransposition

*Ramona Galantonu*, Javier Pizarro, Clément Monot, Nadira Lagha, Margo Montandon, Serdar Kazakyan, Aurore-Cécile Valfort, Pierre-Antoine Vidalain, Gaël Cristofari*

**Institute for Research on Cancer and Aging (IRCAN), UMR CNRS 7284 - INSERM U 1081 - Université Nice Sophia Antipolis, Nice*

Mobile genetic elements play important roles in the evolution and function of the human genome. Among them, the Long Interspersed Nuclear Element-1 (LINE-1 or L1) retrotransposon contributes to the genetic diversity of the human population, and occasionally leads to inherited genetic diseases. L1 elements are also reactivated in many tumors. L1 jumps through a 'copy and paste' mechanism. This process involves two L1-encoded proteins, ORF1p and ORF2p, which associate with the L1 mRNA to form a ribonucleoprotein particle, the core of the retrotransposition machinery. However, little is known about the cellular factors involved in L1 replication. Our laboratory has discovered by yeast 2-hybrid screens that ORF2p, an L1 protein with endonuclease and reverse transcriptase activities, interacts with the estrogen-related receptor α (ERRα), a member of the nuclear receptor family. This observation suggests a model by which ERRα could regulates retrotransposition, possibly by tethering the L1 machinery to chromatin or to specific genomic locations. The existence of several ERRα paralogs prompted us to test whether ORF2p could also interact with other members of this superfamily. To achieve this goal, we used a fluorescent two-hybrid assay (F2H) in mammalian cells. Our results indicate that ORF2p interacts with several other members of the steroid receptors group. To further explore the potential role of this interaction in targeting L1 to chromatin, we artificially tethered ERRα to a unique LacO array and we measure de novo L1 insertions by a cellular retrotransposition assay. Collectively, these data identify steroid signaling pathways as a potential regulatory mechanism for genome instability in human cells.

Poster n°36

# Effect of Seizures on the severity of Epileptic And Cognitive Phenotypes in Mouse Models of Scn1a mutation

*Ana Rita Salgueiro Pereira*, Isabel Delgado-Ruz, Martine Eugie, Ingrid Bethus, Massimo Mantegazza and Hélène Marie*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

The SCN1A gene codes for the α- subunit of the type-I voltage gated sodium channel Nav 1.1. The SCN1A mutation is responsible for genetic epilepsies such as Generalized Epilepsy with Febrile Seizures plus (GEFS+) or Dravet Syndrome (DS). GEFS+ is a milder epilepsy characterized by febrile and afebrile seizure episodes, but no cognitive impairment and in general patients respond well to therapy. DS is a rare, very severe and drug-resistant epileptic encephalopathy (EE). DS children present severe cognitive impairment that, in line with the definition of EE, is caused by the recurrent epileptic activity. However, this definition generate controversy. Nav 1.1 knock-out (KO) mice in the C57bl6/J background show strong epileptic phenotype with striking similarities to DS human disease (Frank, Mantegazza et al. 2006), while this same KO mutant mice in Sv/129 background present a mild phenotype similar to the one found in Nav 1.1, knock-in (KI) mouse, model of GEFS+. The aim of our work is to clarify whether epileptic activity is responsible by itself or not for the cognitive outcome in DS. To assess this question we will two models of Scn1a mutation presenting mild phenotype. We will use a Nav 1.1 KO mouse in Sv/129 background, model of DS and a Nav 1.1 knock-in (KI) mouse in 129:B6 background from F1 generation, model of GEFS+. We will induce seizures by hyperthermia and investigate their effects on epileptic, cognitive phenotypes. Also we will investigate the changes in hippocampal synaptic plasticity caused by recurrent epileptic activity (with field potential recordings in slices). Mutations in Scn1a gene induce increased susceptibility to the occurrence of early-life seizures, but their role in the progression of the disease remains to be clarified. The identification of the pathomechanisms responsible for the disease progression is crucial for development of efficient treatments.

Poster n°37

# Cluster analysis in defining morphological neuronal identity

*Nikita Lukianets*, Michèle Studer, Franck Grammont*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice* and *Laboratoire J.A. Dieudonné, UMR CNRS 7351 - Université Nice Sophia Antipolis, Nice*

The diversity of cell morphology present within a given substructure of the central nervous system reflects a part of its computational complexity. Neuron morphology is a spatial fingerprint that determines organizational principles of the brain by directly influencing connectivity and a signal integration properties. Different parameters of the neuron morphology are commonly used by neuroanatomists as one of the discrimination and classification features of cell populations. For example, characterization of dendritic architecture is important as it represents the input region of the neurons and describes a complex computational unit. Another example of such distinctive features is the soma size, which could define local integration capability, or the number of branching points that directly impact neuron backpropagation properties. In this work, we present a numerical approach using semiautomatic image reconstruction and data clustering techniques to define the morphological identity of different cortical pyramidal cell subclasses. One of our objectives is to study the morphological features of cells co-expressing molecular markers of projection neurons in the somatosensory cortex. Overall, our methodology allowed identifying and linking morphology to molecular identity of cells in a robust and unbiased manner.

Poster n°38

# The Sortilin propeptide : release and functions

*C. Hivelin*, C. Devader, M. Roulot, S. Béraud-Dufour, A. Abderrahmani, J. Mazella and T. Coppola*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

The maturation of the third receptor of Neurotensin (Ntsr3, also called Sortilin) in Golgi apparatus, leads in the release of a propeptide (PE) of 44 residues, which binds the matured Ntsr3 (Kd ~5nM). This PE acts as an antidepressant drug, more efficiently than fluoxetine (Prozac), by blocking the TREK-1 channel. PE is present in mouse blood at a concentration of 20-30nM. The PE blood concentration is correlated with the level of insulin and neurotensin. Indeed, we observed that the injection of insulin (0.75U/kg) in mice induces an increase of PE concentration in the blood. We hypothesized that the PE is released from insulin sensitive organs and can act as a hormone through blood circulation. In order to elucidate its mechanisms of regulation, we use 3T3L1 cells, which can be differentiated into adipocytes. These cells express Glut4, a glucose transporter which stores glucose upon insulin stimulation, and Sortilin, involved in the sorting of Glut4 to the plasma membrane. Glut4 and Sortilin are co-localized in the Golgi apparatus, where the PE is produced. During 3T3L1 cells differentiation into adipocytes, we observed an increase of Glut4 and Sortilin expression at the mRNA and proteins levels. Consequently, the intracellular concentration of PE was also increased. Our laboratory designed a shorter and active form of the PE, of 17 residues, called Spadin and used in the following experiments. In 3T3L1 adipocytes, Spadin doesn't modify the levels of intracellular calcium but stimulates glucose uptake. In vivo, the injection of spadin improves the glucose tolerance. This effect is a consequence of a Spadin-dependent insulin concentration. In vitro, Spadin potentiates the KCl or glucose induced release of insulin in islets. These results suggest the existence of a regulation loop between insulin secreting β cells and insulin sensitive cells, in which the PE would play a central role.

Poster n°39

# Aβ peptide production in Alzheimer's disease : Implication of the unfolded protein response trough the XBP-1s-HRD1 axis

*Yannis Gerakis*, Julie Dunys and Frédéric Checler*

**Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR CNRS 7275 - Université Nice Sophia Antipolis, Sophia Antipolis, Valbonne*

One of the major hallmarks of Alzheimer's disease (AD) is the extra and intracellular accumulation of the amyloïd-β peptide (Aβ) in the brain. This Aβ increase is believed to be linked to the incurable and progressive neurocognitive impairment that is characteristic of the disease. The pathway leading to this Aβ imbalance and the resultant neurotoxicity remain unclear but numerous studies start to report an activation of the unfolded protein response (UPR) in AD patient's brain. Aggregated and misfolded peptides like Aβ are known to be activators of the UPR, yet the possible regulation of Aβ production by the UPR is not very well explored. In this study, we investigate the implication of one main effector of the UPR, the transcription factor XBP-1s, on the Aβ production enzymatic machinery. The Aβ peptide is yielded through the sequential cleavage of its precursor, the amyloid precursor protein (APP), by the β-secretase (Bace1) and γ-secretase. We found that XBP-1s is able to downregulate Bace1 activity and therefore limits Aβ production through the transcription of the ubiquitin-ligase HRD1. HRD1 is already reported to be a regulator of hyper-phosphorylated tau-protein and APP, both implicated in the AD neurodegeneration, thus becoming an interesting target for potential pharmacological treatment. As other members of the UPR, HRD1 is accumulated in AD patient's brain but its activity seems to be disrupted either by its relocation or cellular state. Therefore we sought which mechanism could be able to impair HRD1 regulation axis and then contribute to the Aβ imbalance in AD brain.

Poster n°40

# Transgenerational inheritance of paternal acquired obesity and its associated complications

*Georges Raad*, Valerie Grandjean*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Obesity has exceeded the stage of dietary measures and has reached alerting level worldwide. This is particularly important given that obesity is associated with significant health problem such as development of cardiovascular disease, kidney failure and infertility… Whereas the impact of epigenetic inheritance of obesity on offspring health is well established, the epigenetic adaptative evolution of HFD has not been investigated. The aim of this study is to determine whether High Fat diet feeding in male mice over several generations impacts metabolic health progeny. For that purpose, male mice were were fed a high-fat diet (HFD) from weaning for up to 4 months during 5 successive generations. The resulting offspring was raised on a control diet (CD6). The metabolic phenotype of the CD6 progeny was compared with that of control and CD1 progenies. Strikingly, although the majority of CD1 progeny developed glucose intolerance and insulin resistance, CD6 progenies do not develop those metabolic alterations. By contrast, kidney problems appeared to be more damaged compared to those from control and CD1 progeny. Thus, when compared to control mice, the five generations of male mice on HFD and the generation CD6 showed significant increase in kidney's glomerular area ($p<0.001$), glomerular nuclei ($p<0.001$) and increased accumulation of type IV collagen in glomeruli. Accompanying these histological changes, CD6 mice showed a significant increase in water intake ($p<0.05$), in proteinuria ($p<0.01$) and ketonuria ($p<0.001$). Furthermore, analysis of urine sediment showed that number of crystals is higher in HFD generations and the number of casts is higher in CD6 group compared to control group. In conclusion, this is the first observation of paternal transmission of kidney disease to future generation and could have significant implications for the transgenerational amplification of obesity and related comorbidities worldwide in humans.

Poster n°41

# A dual function of the Zebrafish ESCRT complex in the formation and function of ciliated organs

*Thomas Juan*, Morgane Poulain, Sophie Polès, Marie-Alix Derieppe & Maximilian Fürthauer*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

The Endosomal Sorting Complex Required for Transport (ESCRT) ensures the intra-endosomal sorting of ubiquitinated growth factor receptors en route to lysosomal degradation as well as being involved in membrane-scission during cytokinesis and viral budding. We use the Zebrafish as a model system to study ESCRT function in vertebrate development. Our work reveals that depletion of a Zebrafish ESCRT component causes defects in left/right asymmetry, inner ear development and body axis elongation that are known to be diagnostic of ciliary dysfunction. While cilia of correct number and size are present in ESCRT-depleted animals, our live imaging experiments reveal that ESCRT inactivation specifically impairs ciliary motility. As a consequence of this dyskinesia, ESCRT-depleted animals lack cilia-dependent directional fluid flows and display resulting developmental malformations. In parallel with our discovery of this implication in the regulation of ciliary motility, we have found that multiple ESCRT proteins are strikingly enriched on exovesicles that are present in the lumen of different organs carrying motile cilia. While ESCRTs have been implicated in the extracellular release of viral and exosomal vesicles, little is known about the contribution of ESCRT-positive exovesicles to animal development. We will present data suggesting that ESCRT-positive extracellular carriers contribute to Zebrafish development by ensuring the extracellular transport of signaling molecules and contributing to embryonic morphogenesis. Altogether, our findings suggest that ESCRT proteins contribute to the function of ciliated organs by 1, promoting the creation of cilia-dependent fluid flows and 2, participating in the formation of flow-driven extracellular carrier vesicles.

Poster n°42

# Otx2 controls the proliferation of cerebellar granule cell precursors

*Salsabiel El Nagar*, Nathalie Billon, Thomas Lamonerie*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Transit amplification of granule cell precursors (GCPs) is one of the most important processes of cerebellar development. In a short lapse of time, each precursor will divide symmetrically, in average, eight times, making it prone to mutations which may result in medulloblastoma (MB) formation, the most common pediatric brain tumor. Otx2, a homeodomain transcription factor, is expressed in GCPs throughout their maturation with a posterior-high to anterior-low gradient. Otx2 function in cerebellum development and MB formation is still poorly understood. Otx2 overexpression is observed in 75% of MB. Ectopic expression of Otx2 in medulloblastomas cell lines increases their tumorogenicity, while Otx2 inhibition in xenograft tumors extends animal survival. Moreover, in vitro studies indicate that Otx2 may directly activate cell cycle genes and inhibit differentiation in MB cells. Together, these data suggest that this factor may act as an oncogene in MB formation by driving uncontrolled GCPs proliferation. In this study, we investigated the role of Otx2 in cerebellum development and GCPs proliferation using a conditional Otx2 KO mouse model. This system allows tamoxifen-inducible Otx2 ablation at the time of cerebellum formation (between E16.5 and P21), without affecting early development, where Otx2 plays essential functions. We triggered Otx2 ablation at postnatal day 1 (P1) and analyzed the consequences at P3 and P5. In parallel, we performed a transcriptomic analysis of Otx2 KO cerebellum to identify Otx2 targets in this organ. We show that Otx2 invalidation consistently results in a reduction of the posterior cerebellum size and in a decrease in the number of proliferating GCP. We are now assessing the function of Otx2 in a murine model of MB. Understanding the role and identifying the targets of Otx2 in normal and tumoral cerebellar GCPs will allow to develop new therapies to fight against MB.

Poster n°43

# Is the immunotherapy with Fractalkine an attractive strategy for bone metastases?

*Rasha Al-Sahlanee*, Charlotte Cohen, Emilie Goguet-Surmenian, Millet Marie-Ange, Heidy Schmid-Antomarchi, Annie Schmid-Alliana*

**Institut de Biologie Valrose (IBV), UMR CNRS 7277 - INSERM U 1091 - Université Nice Sophia Antipolis, Nice*

Bone is a common metastatic site for many types of cancers. Despite recent therapeutic advances, the bone metastases still remain incurable, prompting the research of new therapeutic strategies, such as immunotherapy.In this context,the chemokines,considered as key players of the immune antitumor response, appear as potential tools of the anticancer immunotherapy.

CX3CL1 chemokine (FKN) is able to recruit/activate essential actors of antitumor response which can counteract the tumor development. However, FKN can also facilitate cancer advance by promoting tumor angiogenesis and/or stimulating adhesion and migration of cancer cells. Moreover, FKN is described to positively control osteoclastic activity and thus, could promote the tumor growth in the bone.

The objective of this thesis is to define if the FKN-CX3CR1 axis is in fact an attractive curative strategy in bone cancer.

To do so, the pro/anti-tumor effect of FKN in bone, we have developed, in mice, two models of bone metastases using genetically modified cancer cells to produce FKNs.Thereby, we showed that the expression of FKN inhibit the development of lung cancer cells in the bone. This inhibition is correlated with an increase in the leukocyte recruitment into tumors. qPCR analysis of tumors revealed that the FKN impacts on bone balance in particular by modulating the balance\""OPG/RANK/RANKL\"" and the BMP expression in favor of osteogenesis. Thus, in this model of bone metastasis where tumor cells do not express CX3CR1, it seems to be beneficial to promote the CX3CR1/FKN axis to inhibit tumor development. Given the direct effects of FKN on tumor cells, it was essential to study the effects of FKNs on bone development of cells expressing CX3CR1. Thus, by using renal cancer cells, which meets this criterion, we observed that the expression of FKN stimulates their development in the bone. qPCR analysis of these tumors showed that FKN also control the bone balance but in this case to promote osteolysis.

# PROGRAM

## AT A GLANCE

| TIME | THURSDAY, SEPTEMBER 3RD 2015 | FRIDAY, SEPTEMBER 4TH 2015 |
|---|---|---|
| 07:30 | | |
| 07:45 | 07:30-08:00 Registration | |
| 08:00 | | |
| 08:15 | 08:00-08:15 Opening speech (Dr. Thomas LAMONERIE) | 08:00-08:30 Registration |
| 08:30 | | |
| 08:45 | | |
| 09:00 | 08:15-09:30 Oral Communications - Session 1 | 08:30-09:30 Oral Communications - Session 5 |
| 09:15 | | |
| 09:30 | | |
| 09:45 | | 09:30-10:30 Keynote Lecture - Dr. Lorenzo GALLUZZI |
| 10:00 | 09:30-10:30 Oral Communications - Session 2 | « Unsaturated fatty acids induce non-canonical autophagy » |
| 10:15 | | |
| 10:30 | | |
| 10:45 | 10:30-11:00 Coffee Break | 10:30-11:00 Coffee Break |
| 11:00 | | |
| 11:15 | | |
| 11:30 | 11:00-11:45 Oral Communications - Session 3 | 11:00-12:00 Round Table with UNICEPRO |
| 11:45 | | |
| 12:00 | 11:45-12:00 My Poster in 30 seconds - Session 1 | |
| 12:15 | | 12:00-12:15 My Poster in 30 seconds - Session 2 |
| 12:30 | 12:00-13:00 Keynote Lecture - Dr. Bart STAELS | |
| 12:45 | « Circadian control of metabolism: Rev-erbs as potential therapeutic targets » | 12:15-13:00 Lunch Break |
| 13:00 | | |
| 13:15 | 13:00-14:30 Lunch Break | |
| 13:30 | | |
| 13:45 | | |
| 14:00 | | 13:00-14:30 Poster Session |
| 14:15 | 14:30-15:00 Poster Session | |
| 14:30 | + Workshop | |
| 14:45 | « Career opportunities in a biotechnology company » | |
| 15:00 | | |
| 15:15 | | 14:30-15:45 Oral Communications - Session 6 |
| 15:30 | 15:00-16:00 Keynote Lecture - Dr. Marie MIROUZE | |
| 15:45 | « The epigenome : a guardian of the plant mobilome? » | |
| 16:00 | | 15:45-16:45 Oral Communications - Session 7 |
| 16:15 | 16:00-16:30 Coffee Break | |
| 16:30 | | |
| 16:45 | | |
| 17:00 | 16:30-17:30 Oral Communications - Session 4 | 16:45-17:15 JEDNs Awards |
| 17:15 | | |
| 17:30 | | |
| 17:45 | | |
| 18:00 | 17:30-19:00 Drinks | |
| 18:15 | | |
| 18:30 | | |
| 18:45 | | |
| 19:00 | | |
| 19:15 | | |
| 19:30 | | |
| 19:45 | | |
| 20:00 | | |
| 20:15 | | |
| 20:30 | 19:00-22:00 Dinner | |
| 20:45 | | |
| 21:00 | | |
| 21:15 | | |
| 21:30 | | |
| 21:45 | | |
| 22:00 | | |