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Journées de l'École Doctorale de Nice

Université Nice Sophia Antipolis

ED85 Sciences Vie Santé

4 et 5
Septembre 2014
Faculté des Sciences
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14ÈMES JOURNÉES DE L'ÉCOLE DOCTORALE DE NICE

PROGRAMME SCIENTIFIQUE

TABLE DES MATIERES

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Professeur au Muséum National d'Histoire Naturelle (Paris) et ancien professeur de l'Université Montpellier II.

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Chef de l'équipe « Immunité Pulmonaire », Centre Infection et Immunité, Lille.

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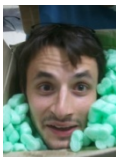

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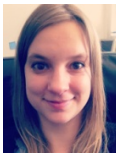

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PROGRAMME DÉTAILLÉ

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CCL17 production by dendritic cells is required for NOD1-mediated exacerbation of asthma

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SESSION POSTER

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SESSION POSTER

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RÉSUMÉS DES CONFÉRENCES PLÉNIÈRES

CCL17 production by dendritic cells is required for NOD1-mediated exacerbation of asthma

Anne Tsicopoulos
Pulmonary Immunity, U1019, Center for Infection and Immunity of Lille, Institut Pasteur de Lille, France.

Pattern recognition receptors are attractive targets for vaccine adjuvants and among them polymorphisms of the innate receptor NOD1 have been associated with allergic asthma. Adjuvanticity elicited by systemic administration of NOD1 and NOD2 agonists stimulate splenic Th2- mediated responses in mice, a response observed in asthma. However, it is unknown whether it may favor allergic diseases in humans. We show herein that NOD1-mediated conditioning of human dendritic cells (DCs) promoted a Th2 cell polarization profile which involved the production of both CCL17 and CCL22 in nonallergic subjects but only CCL17 in allergic patients, without requiring allergen co-stimulation. Moreover, NOD1-primed dendritic cells from allergic donors exhibited enhanced maturation that led to abnormal CCL22 and IL-10 secretion compared with nonallergic donors. In mice, systemic NOD1 ligation exacerbated allergen-induced experimental asthma by amplifying CCL17-mediated Th2 responses in the lung. Interestingly, NOD1-mediated sensitization of purified murine DCs enhanced production of CCL17 and CCL22, but not of TSLP and IL-33 in vitro. Consistently, adoptive transfer of NOD1-conditioned DCs exacerbated the Th2 pulmonary response in a CCL17-dependent manner in vivo. Altogether these data unveil a predominant role of NOD1 in allergic asthma through direct activation of DCs and induction of CCL17, arguing for a need to address vaccine formulation safety issues related to allergy, to prevent an increase in the burden of allergic diseases.

Mycoheterotrophy and mixotrophy: plants eating mycorrhizal fungi.

Marc-André Selosse
Muséum national d'Histoire naturelle, Département Systématique et Evolution (UMR 7205 OSEB), CP 50, 45 rue Buffon, 75005 Paris, France;

The evolution of land plants provided repeated emergences of mycoheterotrophy, where achlorophyllous plants exploit carbon from their mycorrhizal fungi. This condition, suggested to be an adaptation to forest environments where little light is available, recently made strong achievements due to two tools: fungal molecular barcoding allowed identification of the (often uncultivable) mycorrhizal fungi; natural isotopic abundances supported which fungal guild was giving carbon to the mycoheterotrophic plants. Temperate and Mediterranean species, mainly orchids and Montropoideae (Ericaceae), have specific basidiomycetous fungal partners that usually form mycorrhizae with surrounding trees (ectomycorrhizal fungi). By contrast, subtropical and tropical species often connect to arbuscular-mycorrhizal (AM) fungi or even to saprotrophic, wood- or litter-decaying basidiomycetes. Their specificity is often lower.

More recently, intermediate evolutionarily steps were found to exist, where the plant is still green and photosynthetic, but partly uses carbon from its fungal associates. This strategy,

called mixotrophy, is now well described for green temperate orchids and Montropoideae species associated to ectomycorrhizal basidiomycetes or sometimes ascomycetes. Phylogenetic frameworks suggest that mixotrophy pre-dispose to evolution of mycoheterotrophy. In some mixotrophic *Cephalanthera* and *Epipactis* spp. (orchids), the rare survival of achlorophyllous plants (albinos) further supports their use of fungal carbon. More recently, our investigations of albinos' nutrition and fitness revealed why emergence of mycoheterotrophy is rare in evolution of mixotrophs: photosynthesis is not used for survival, which is supported by fungal carbon, but mainly for seed production. Thus, photosynthesis loss drastically reduces fitness and, as a result, mixotrophy is evolutionarily metastable.

Dietary triglycerides act on mesolimbic structures to regulate the rewarding and motivational aspects of feeding

C Cansell[1], J Castel[1], RGP Denis[1], C Rouch[1], A-S Delbes[1], S Martinez[1], D Mestivier[2], B Finan[3], JG Maldonado-Aviles[4], M Rijnsburger[5], MH Tschöp[3,6], RJ DiLeone[4], RH Eckel[7], SE la Fleur[5], C Magnan[1], TS Hnasko[8] and S Luquet[1]

[1]Unité de Biologie Fonctionnelle et Adaptative, Sorbonne Paris Cité, CNRS UMR 8251, Université Paris Diderot, Paris, France; [2]Institut Jacques Monod, Sorbonne Paris Cité, Université Paris Diderot, Paris, France; [3]Helmholtz Diabetes Center, Helmholtz Zentrum München, German Research Center for Environmental Health, München/Neuherberg, Germany; [4]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA; [5]Department of Endocrinology and Metabolism, University of Amsterdam, Amsterdam, The Netherlands; [6]Division of Metabolic Diseases, Department of Medicine, Technische Universität München, München, Germany; [7]Division of Endocrinology, Metabolism and Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO, USA and [8]Department of Neurosciences, University of California, La Jolla, CA, USA.

Circulating triglycerides (TGs) normally increase after a meal but are altered in pathophysiological conditions, such as obesity. Although TG metabolism in the brain remains poorly understood, several brain structures express enzymes that process TG enriched particles, including mesolimbic structures. For this reason, and because consumption of high-fat diet alters dopamine signaling, we tested the hypothesis that TG might directly target mesolimbic reward circuits to control reward-seeking behaviors.

We found that the delivery of small amounts of TG to the brain through the carotid artery rapidly reduced both spontaneous and amphetamine-induced locomotion, abolished preference for palatable food and reduced the motivation to engage in food-seeking behavior. Conversely, targeted disruption of the TG-hydrolyzing enzyme lipoprotein lipase specifically in the nucleus accumbens increased palatable food preference and food-seeking behavior. Finally, sustained hypertriglyceridemia achieved through prolonged TG perfusion diet-induced obesity resulted in a return to normal palatable food preference despite continued locomotor suppression, suggesting that adaptive mechanisms occur. These findings reveal new mechanisms by which dietary fat may alter mesolimbic circuit function and reward seeking.

Spatial ecology of seabirds; from microelectronics to the conservation of the species

David Grémillet
Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier

Human activities strongly affect marine ecosystems. It is essential to understand these impacts and to mobilize public opinion in order to achieve practices more respectful for the environment. In this context, seabirds appear as flagship species for three main reasons. First, they are of interest because of their role in marine trophic networks, as well as their other participants (fish, plankton, viruses). Secondly, 29% of seabird species are threatened, more than any other bird group. It is therefore essential to study them in order to better preserve them. Finally, seabirds are emblems of marine conservation; their aura can transform our ways of thinking regarding the marine world. Despite these issues, the movements of seabirds at sea have long remained a mystery. The new electronic technologies allow, since 20 years, a revolution in marine ecology. Throughout this presentation I will detail how these technological advances allow researchers to explore the spatial ecology of seabirds, from their small-scale movements within a breeding colony, to their planetary migration. This trip will take us to my favorite areas of research in the Mediterranean, in southern Africa and tin he Arctic, for studies to test the impact of climate changes and fisheries on seabirds in order to propose new measures to manage marine biodiversity such as marine protected areas.

Long-term experiment with *Escherichia coli*: is evolution repeatable?

Dominique Schneider
Laboratoire « Adaptation et Pathogénie des Microorganismes », Equipe « GEM - Génomique et Evolution des Microorganismes », Grenoble

Systems Biology, through its interdisciplinary nature, highlighted the multifaceted complexity of living organisms by improving our understanding of the structure and function of genomes and cellular networks. Integrating evolutionary perspectives is fully complementary by providing a dynamic view of virtually all cellular processes. Experimental evolution is designed to reproduce evolution in controlled laboratory conditions and therefore provides such an evolutionary framework.

During the longest running evolution experiment, twelve populations of *Escherichia coli* are independently propagated from a common ancestor in a defined environment for more than 55,000 generations. The full revivable fossil record of the entire evolution experiment is investigated for phenotypic, genomic and global expression changes. All populations achieved substantial fitness improvement during evolutionary time. Adaptive changes have been shown to be associated with complex effects on global gene expression, including widespread pleiotropy and epistasis, indicative of important changes in regulatory networks.

The identified changes are highly parallel, with similar phenotypes and networks being repeatedly modified in most populations. This high level of phenotypic and genetic parallelism might be integrated in predictive models of bacterial behaviour. However, two lines of

evidence suggest that predictions might be more difficult than expected. First, the evolved bacterial genomes revealed an exceptional dynamics in their mutation rates, reflecting a tension between adaptation and genetic load that is strongly related to the fit of the bacterial populations to their environment. Second, a unique diversification event emerged in one population, associated with allelic specificity and epistatic interactions among mutations.

RÉSUMÉS DES PRÉSENTATIONS ORALES

Oral tolerance is inefficient in neonates due to physiological vitamin A deficiency

Mathilde Turfkruyer, Akila Rekima, Patricia Macchiaverni and Valérie Verhasselt*

**EA6302, L'Archet 1, Nice*

Heightened sensitivity to allergic disease in early life draws attention on possible deficient immune regulation in addition to well-described inadequate effector immune responses during this period of life. We found that oral tolerance induction in breastfed mice was not efficient until their third week of life. Inability of neonate to develop oral tolerance was a consequence of defective antigen uptake and retinaldehyde deshydrogenase expression by mesenteric lymph node CD103+ DC which resulted in inefficient T cell activation and antigen ignorance. Physiological low levels of vitamin A in the neonate were found to be responsible for such defects and supplementation with vitamin A during neonatal period was sufficient to allow oral tolerance induction from first week of life. Finally, we found that oral tolerance in vitamin A supplemented-neonates and 3 week old pups were critically dependent on IFN-γ while Foxp3 Tregs and Tr1 are known to mediate oral tolerance in adults. The identification of mechanisms underlying efficiency of induction of a key process for immune homeostasis should help prevent immune disease in early life.

Genetic Disruption of Lactate-H+ Symporters (MCTs) and their Subunit CD147/Basigin Sensitizes Glycolytic Tumor Cells to Phenformin

Ibtissam Marchiq, Renaud Le Floch, Danièle Roux, Marie-Pierre Simon and Jacques Pouysségur*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Introduction: To achieve robust glycolytic rate, rapidly growing tumors have to maintain intracellular pH (pHi) and therefore must efficiently export lactic acid. Two major MonoCarboxylate Transporters, MCT1 and the hypoxia-inducible MCT4, complexed with the ancillary glycoprotein CD147/Basigin, execute this function as bidirectional H+/ lactate- symporters.

To further explore i) the physiology of MCTs/Basigin complexes and ii) validate the blockade of lactic acid export as an anticancer strategy, we genetically disrupted, via Zinc Finger Nucleases, mct4 and basigin genes in two human cell lines of colon adenocarcinoma (LS174T) and Glioblastoma (U87).

Results: First, we showed that single ablation of mct4 gene is not sufficient to alter tumor cell metabolism and growth. However, it dramatically sensitizes cells to AZD3965 the pharmacological inhibitor of MCT1. Second, we demonstrated that knockout of basigin (bsg) gene reduced the plasma membrane expression and lactate transport activity of both MCT1 and MCT4 by respectively 10- and 6- fold. As a consequence of this decrease, cells accumulated an intracellular pool of lactic and pyruvic acids magnified by the further addition of MCT1 inhibitor, an action decreasing further pHi and the rate of glycolysis. Thirdly, we found that these glycolytic/MCT-deficient cells survived and resumed growth by redirecting part of their energy metabolism towards oxidative phosphorylation. Finally, we showed that in contrast to tumor parental cells, their derivatives basigin-/- or basigin-/- and mct4-/- became highly sensitive to phenformin, an inhibitor of mitochondrial complex I. Phenformin addition to these MCT-disrupted cells in normoxic and hypoxic conditions induced a rapid and major drop in cellular ATP provoking cell death by « metabolic catastrophe ».

Conclusion: Taken together, these findings highlight that MCTs/Basigin complexes inhibition, combined with phenformin provide a novel acute anticancer strategy for highly glycolytic tumors. This genetic approach validates the anticancer potential of MCT1 and MCT4 inhibitors in current development.

Role of the JNK signaling pathway in cell reprogramming

S. Roumengous, R. Rousset, S. Noselli*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

The JNK signaling pathway is implicated in a multitude of diseases ranging from cancer to neurological and immunological/inflammatory conditions in which an abnormal activation of it is observed. This pathway, evolutionary conserved, is activated during Drosophila embryo's dorsal closure, to induce an unexpected cell reprogramming event in the ectoderm. My works reveal a link between reprogramming and PcG (Polycomb Group) genes, a family of silencing genes acting on chromatin structure. In contrast to expectations, Polycomb, a gene belonging to PcG genes family, shows a positive role on this specific process, leading to the characterization of innovative mechanisms acting on cellular reprogramming during normal development.

Anti-proteases target cancer stem cells expressing an embryonic signature and decrease their tumour potential

P. Martin, C.Y. Darini, S. Azoulay, M-D. Drici, P. Hofman, C.Dani, A. Ladoux*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Objective: Cancer stem cells (CSCs) constitute a specific subset of the cancer cell population in the majority of solid tumours, where they contribute to the chemoresistant phenotypes characteristic of many cancer types. They play a key role in self-renewal sustaining tumour growth and metastasis. Among them, CSCs displaying an embryonic stem cell « stemness » signature based on the expression of Oct-4, Nanog and Sox2, are present in distinct high grade tumour types associated with poor prognosis. In this study, molecules targeting them specifically were identified as potential therapeutic agents which might reduce malignant progression, then preventing tumour recurrence.

Material: We previously set up a model to isolate pure populations of CSCs expressing an embryonic signature from distinct solid tumours. They were isolated from spontaneously developing solid tumours obtained from p53-/- mice expressing GFP and the puromycin resistance gene under the control of the Oct-4 promoter. Pure populations of Oct-4-expressing cells were obtained after puromycin selection. Molecules were screened to specifically target them and reduce their proliferation as compared to the total population of cancer cells isolated from the same tumour. Cell death induction was assessed for candidate molecules. Then, the efficiency to impair CSCs tumour potential was evaluated by allograft formation assays in immune-deficient mice.

Results: We show that HIV-protease inhibitors (HIV-PIs) specifically target CSCs expressing an embryonic signature. They reduced proliferation in a dose-dependent manner with higher specificity and efficiency as compared to the total population of cancer cells and/or healthy stem cells. Moreover, they were efficient to induce cell death. Lopinavir (LPV) was the most effective HIV-PIs among those tested. Structure-activity relationship experiments performed using key intermediates for LPV synthesis allowed the identification of essential pharmacophores for LPV-antitumour specificity and activity. LPV-induced CSCs death was accompanied by the expression of activated-caspase 3 and cleavage of the DNA repair enzyme poly (ADP-ribose) polymerase: PARP-1 which represents a hallmark of apoptosis. In addition, in vivo treatment of mice with a fixed association of lopinavir and ritonavir according to the standard posology recommended for humans, resulted in a reduction of allografts formation, indicating a beneficial effect on tumour regression.

Conclusions: These results contribute to the identification of molecules presenting selective toxicity for CSCs expressing an embryonic stemness signature. This offers promising therapeutic opportunities for patients suffering from solid cancer tumours of poor prognosis.

BCL-B (BCL2L10) is overexpressed in patients suffering multiple myeloma (MM) and drives MM-like disease in transgenic mice

Mohamed-Amine Hamouda, Alexandre Puissant, Guillaume Robert, Arnaud Jacquel, Emmanuel Griessinger, Diogo Goncalves, Sandrine Roulland, Aude Mallavialle, Pascal Colosetti, Sandrine Marchetti, Martine Amiot, Nathalie Rochet, Marcel Deckert, Paul Hofman*

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

Multiple Myeloma (MM) evolves from a premalignant condition known as monoclonal gammopathy of undetermined significance (MGUS). However, the factors underlying the malignant transformation of plsmocytes in MM are not fully characterized. Here, we report an MM phenotype in transgenic mice with Eμ-directed expression of the Bcl-B (BCL2L10) protein, an anti-apoptotic member of the Bcl-2 family. With age, Eμ-Bcl-B transgenic mice develop the characteristic features of human MM, including indolent malignant plasma cell expansion restricted to the bone marrow, monoclonal gammaglobulinemia, anemia, bone lytic lesions and renal immunoglobulin deposition. in addition, the MM-like disease was serially transplantable in irradiated receiving mice, underlying the tumoral origin of mice plasmocytes. Furthermore, the transcriptional profiles of Eμ-Bcl-B bone marrow B cells show increased expression of genes that are known to be dysregulated in human MM. Importantly, Bcl-B is overexpressed in purified bone marrow CD138+ cells from MM patients but not from patients with MGUS or healthy individuals, wich strongly suggests that Bcl-B drives MM. The similarities of this model to the human disease, together with Bcl-B overexpression in human MM, identify this protein as an essential factor in MM pathogenesis.

Incorporation of chemical element gallium enhances biological properties of calcium phosphate biomaterials for bone reconstruction

I Strazic, N Melis, F Boukhechba, C Mellier, P Janvier , J Lesoeur, X Mouska, E Goguet-Surmenian, N Rochet, J-M Bouler, E Verron, JC Scimeca.*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Calcium phosphate-based biomaterials are commonly used in bone reconstructive surgery, and if combined with active compounds, can serve as a vector for local drug delivery. Gallium (Ga) has been used in treatment of several disorders associated with accelerated bone mineral resorption, and it was shown to inhibit the differentiation of osteoclasts, the bone-resorbing cells. It was also demonstrated that Ga can be incorporated in the structure of calcium phosphate (CaP) following an ionic substitution of calcium sites. In the present work we embarked on investigating biological properties of novel gallium-substituted CaP biomaterials.

In vitro, human primary osteoblasts and human primary monocytes were cultured together on pellets made from control or Ga-substituted biomaterials. In this co-culture model, osteoblastic signals allow monocytes to differentiate into mature osteoclast and at day 14, tartrate-resistant alkaline phosphatase (TRAP) staining revealed mature osteoclasts on both biomaterials. Furthermore, a decrease in osteoclasts number was observed on the surface of Ga-substituted pellets compared to control biomaterial, potentially suggesting an inhibition of osteoclastic differentiation. Using a rat model of bone defect healing, we next investigated in vivo the bone reconstructive properties of Ga-substituted biomaterials. Briefly, cylindrical defects in rat femora were implanted with control or Ga-substituted biomaterial micro-particles. For all tested biomaterials, we found a good osseointegration of implants into the surrounding host tissue accompanied by successful bone ingrowth and bone marrow reconstruction, as observed by histological staining and scanning electron microscopy. Moreover, 3D quantitative micro-CT analysis showed at 2 months a higher percentage of newly formed bone tissue in the presence of Ga compared to control condition.

Taken together, our data indicate that control or Ga-substituted biomaterials provide biocompatible and non-cytotoxic substrates for bone cells survival in vitro, thus allowing for their functional dialogue and osteoclastic differentiation. A decrease in mature osteoclasts number on Ga-substituted pellets suggests an inhibition of osteoclastic differentiation. Preliminary results in our in vivo model imply that gallium presence in the structure of CaP biomaterials can potentially have a beneficial effect on bone remodeling in defect healing.

Involvement of FMRP sumoylation in the control of spinogenesis

Anouar Khayachi, Frédéric Cassé, Gwénola Poupon, Carole Gwizdek, Barbara Bardoni and Stéphane Martin*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

Post-translational modifications play essential roles in many aspects of cellular functions and in the maintenance of cell integrity. Sumoylation is a post-translational modification that consists in the covalent but reversible conjugation of the Small Ubiquitin like MOdifier protein SUMO to specific lysine residues of target proteins. Sumoylation is a well-characterized regulator of nuclear functions but also emerges as a key factor for numerous extranuclear processes. Interestingly, it was demonstrated that sumoylation plays key roles in neuronal development and synaptic plasticity. We recently identified a protein shown to be important for the regulation of neuronal morphology that is a target for sumoylation in vivo. To unravel the role of this protein sumoylation on spine architecture we used site-directed mutagenesis to engineer a non-sumoylatable target protein. Using viral transduction and confocal microscopy, we assessed the neuronal morphology and synaptic architecture from primary cultured neurons expressing the WT or non-sumoylatable form of the protein. Our data indicate that overexpression of the non-sumoylatable protein in neurons is able to disrupt neuronal morphology indicating that it acts as a dominant negative on the endogenously expressed target protein. Here we demonstrate that sumoylation is important for the control of spine morphology.

A phosphatidylinositol-4-phosphate powered exchange mechanism to create a lipid gradient between membranes

Moser von Filseck, J., Vanni, S., Mesmin, B., Antonny, B., Drin, G.*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

In eukaryotic cells, sterols are transported to late membranes at the expense of the ER, where sterol is synthesized, along non-vesicular routes whose nature remains elusive. Recently, we revealed that Osh4p, a member of the oxysterol-binding protein (Osh/ORP) family can exchange sterol for PI(4)P between membranes. Therefore, we posit that Osh4p ensures an anterograde transport of sterol at the ER/Golgi interface by exploiting the PI(4)P gradient established by two spatially distant enzymes: the Golgi PI4-kinase Pik1p and the ER-resident PI(4)P phosphatase Sac1p. This might explain how a carrier can create an intracellular sterol gradient. To further support this hypothesis, we measured with an unprecedented accuracy, using novel real-time assays, the sterol/PI(4)P exchange activity of Osh4p between ER- and Golgi-mimicking membranes.

Osh4p quickly exchanges sterol for PI(4)P between membranes until full equilibration of PI(4)P. The hydrolysis of PI(4)P by Sac1p on ER-like liposomes maintains the PI(4)P gradient, sustaining the sterol transfer by Osh4p. Strikingly, Osh4p can efficiently release sterol into Golgi-like membranes even against a pre-existing sterol gradient. Sterol sequestration in these membranes by saturated lipids and sphingolipids amplifies this activity. The equal affinity of Osh4p for sterol and PI(4)P and its ability to specifically control the release of each lipid are key for its activity. Thus, we provide the first demonstration that Osh4p is likely a genuine sterol carrier able to transport sterol against its concentration gradient under the control of PI(4)P to maintain sterol homeostasis in cells.

Dual role of a Notch pathway component in epithelial morphogenesis

Priyanka Sharma and Maximilian Fürthauer*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

A remarkable feature of epithelial tissue is that it exhibits polarity on cellular and tissue levels namely apico-basal polarity and planar cell polarity respectively. We aim to understand epithelial morphogenesis in the context of the zebrafish neural tube. Notch signaling which is one of the major signaling pathways and of prime importance in neurogenesis has been widely studied for its function in cell fate specifications in the central nervous system. We found that in addition to this well-known function, one of the components of Notch signaling plays a dual role in the neural-tube morphogenesis by affecting both cellular and tissular polarity. On one hand, it participates in Notch dependent regulation of apico-basal polarity of the neuroepithelium. The establishment of apico-basal polarity completely diminishes upon failure of transcriptionally mediated canonical Notch signaling. On the other hand, we have identified a surprising new function of a Delta/Notch signaling component in the regulation of the morphogenetic movements that shape the zebrafish spinal cord. This is achieved by regulating planar cell polarity to control convergent-extension movements, independent of its role in Notch signaling. Overall, our study presents a novel link between apico-basal and planar cell polarity which will allow us to understand further epithelial morphogenesis in the zebrafish neural tube development.

HMGA2 deregulation in adipocytic tumors

Esma Saada, Laurence Bianchini, Florence Pedeutour*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Adipocytic tumors (AT) are the most frequent human mesenchymal tumors. Cytogenetic analysis helps to distinguish benign (as ordinary lipomas) and well differentiated tumors (atypical lipomatous tumors, ALT) or to assess the diagnosis of dedifferentiated liposarcoma (DDLPS) when the histological diagnostic is uncertain. Molecular characterization of AT is one of the main research axis of our laboratory.

HMGA2 gene (High Mobility Group A2) is localized on the long arm of the chromosome 12 (12q14.3) and codes for a non-histone chromatin protein. The first three exons codes for structural DNA-binding domains. HMGA2 is expressed in human early development and is undetectable in normal adult tissues. The 3' part of HMGA2 mRNA contains binding sites for let-7 miRNA that negatively regulate HMGA2 transcription. HMGA2 is structurally rearranged in a majority of ordinary lipomas. Recent works in our laboratory have shown that the three first exons of HMGA2 were also systematically amplified in a series of 38 ALT/DDLPS suggesting a crucial role for HMGA2 in AT pathogenesis.

We first studied HMGA2 regulation in lipomas and the role of let-7 miRNA in lipoma biology. HMGA2 mRNA was overexpressed in 100% of lipomas with structural rearrangement and 76% of lipomas without rearrangement, suggesting a different mechanism of deregulation for those tumors. We studied the expression of 8 let-7 family members and did not find any significant correlation between let-7 expression and HMGA2 one. Thus, we invalidated the hypothesis of a let-7 mediated deregulation of HMGA2 expression in lipomas.

We also analyzed a case of lipomatosis, a rare proliferative disorder of the adipocytic tissue. In the absence of structural rearrangement of HMGA2, HMGA2 mRNA and protein overexpression were associated with an inhibition of all of the analyzed let-7s, suggesting a role for let-7 in HMGA2 regulation in lipomatosis.

We then studied the role of HMGA2 fusion partners in rearranged lipomas. Indeed, the overexpression of HMGA2 in those lipomas may be due to the loss of negatively acting domains located in the 3'UTR or the complementary action of the partner of HMGA2. Though more than 40 regions have been reported, only 5 partner genes have been identified so far. In a series of lipomas with t(1;12), we characterized a 1p32 recurrent breakpoint by means of FISH-based positional cloning. We identified PPAP2B, a member of the lipid phosphate phosphatases family as the target gene. Reverse transcription-PCR analysis followed by nucleotide sequencing of the fusion transcript indicated the fusion between HMGA2 and PPAP2B in one case.

Finally, we studied by FISH series of 117 ALT/DDLPS which are characterized by MDM2 gene constant amplification. The exact role and the frequency of amplification of HMGA2, CDK4 or JUN are not well established. The three first exons of HMGA2 were amplified in 88% of tumors, which was statistically linked with good prognosis factors and adipocytic differentiation. CDK4 and JUN amplification, detected in 69% and 42% of tumors respectively, were associated with dedifferentiated subtype and worse prognosis features. Our original results confirm the early role of HMGA2 in ALT-DDLPS genesis and the secondary place of JUN and CDK4 that may act as added genetic alterations.

Diagnosis and prognosis performances of different anti-PLA2R1 assays

*Barbara Seitz-Polski**

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

Idiopathic membranous nephropathy (iMN) is an auto-immune disease and a common cause of nephrotic syndrome in adults that leads in 30% of case to end stage kidney disease with starting hemodialysis or kidney graft. About 70% of patients with idiopathic membranous nephropathy (iMN) have circulating autoantibodies against the phospholipase A2 receptor PLA2R1. These antibodies seem to correlate with disease activity but some patients had persistent antibodies in remission. There is a need for the detection of diagnostic parameters that would allow the identification of patients at risk of poor clinical outcome and who will benefit from immunosuppressive.

We screened iMN patients positive for anti-PLA2R1 for their cross-reactivity against human (h), rabbit (rb) and mouse (m) PLA2R1, first by western blot, then using antigen-specific ELISAs. All patients recognized almost equally hPLA2R1 and rbPLA2R1 by western blot, and a rbPLA2R1 ELISA was as performant as the standardized hPLA2R1 ELISA to monitor anti-PLA2R1 in iMN. We used this test to show in a cohort of 15 patients with kidney graft after MN that monitoring of anti-PLA2R1 Ab after transplantation can predict MN recurrence.

In contrast, only 51% of patients were cross-reactive against mPLA2R1 by western blot, revealing that iMN patients exhibit different subsets of anti-PLA2R1 autoantibodies against epitopes that are shared or not among the three PLA2R1 orthologs. In a cohort of 41 patients with a follow-up of >36 months, the detection of anti-mPLA2R1 antibodies at first sample analysis was an independent predictor of clinical outcome in multivariate analysis (p=0.02), and ROC curve analysis identified a threshold of 605 RU/mL above which 100% of patients (12 patients) had a poor renal outcome (p=0.002). To confirme this result we performed western blot recognition of 50 sera from iMN patients with anti-PLA2R1 Ab using 10 deletion mutants of PLA2R1 produced in HEK cell. We identify at least 3 different epitopes implicated in PLA2R1 Ab recognition: one epitope in N-terminal domain is associated with less active disease and good prognosis. We identified an intra-molecular epitope spreading in patients who modified disease activity.

Our results indicated that PLA2R1 epitope profile may help to predict the need for immunosuppressive treatment and subsequent renal outcome.

The P2Y6-AMPK pathway triggers autophagy during monocyte differentiation: A potentiel target for therapeutic intervention in CMML

Sandrine Obba, Zoheir Hizir, Dorothée Selimoglu-Buet, Laurent Boyer, Anja Pfeifer, Mohamed-Amine Hamouda, Diogo Gonçalvès, Michael Cerezo, Sandrine Marchetti, Stephane Rocchi, Nathalie Droin, Guillaume Robert, Frederic Luciano, Benoit Viollet, Laurence Le*

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

Characterizing the physiological differentiation of monocytes into macrophages is essential to understand the pathophysiology of Chronic MyeloMonocytic Leukemia (CMML). Indeed, monocytes from patients suffering from CMML are unable to differentiate into macrophages, resulting in a monocytosis that is characteristic of this disease. Differentiation of monocytes into macrophages is governed by, Macrophage-Colony Stimulating Factor (CSF-1) and may be reproduced ex vivo using isolated peripheral blood monocytes treated with CSF-1. In this context, we previously established that autophagy is required for CSF-1-induced macrophagic differentiation and acquisition of phagocytic functions. This autophagy is dependent on the ULK1-Atg13-FIP200 complex. However, little is known about the molecular mechanisms that link CSF-1 receptor engagement to the induction of autophagy.

Our works demonstrate, for the first time that the CaMKKβ-AMPKα1-ULK1 pathway is required for CSF-1-induced autophagy and during a context of differentiation.

Moreover, this pathway links P2Y6 to the induction of autophagy, and we decipher the signalling network that links the CSF-1 receptor to P2Y6-mediated autophagy and monocyte differentiation. In addition, the physiological P2Y6 ligand UDP and the specific P2Y6 agonist MRS2693 restore normal monocyte differentiation through re-induction of autophagy in primary myeloid cells from chronic myelomonocytic leukemia (CMML) patients. Collectively, our findings highlight an essential role for P2Y6-mediated autophagy during differentiation of human monocytes and pave the way for future therapeutic interventions for CMML.

Thyroid Function Disruption: Setting up a screening strategy for chemicals

Constantin Dallot et Anny Cupo*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

There is currently a lack of *in vitro* models suitable for the early detection of the perturbation of thyroid function. The aim of this work was to explore the possibility to detect the perturbation of the hormone synthesis and metabolism using RT-qPCR, respectively in a thyrocyte cell line and in alginate embedded cryopreserved hepatocytes.

Thyroid hormone synthesis occurs in thyroid follicles and involves specific proteins. Interference with the hormone synthesis can lead to the deregulation of the transcription of the corresponding genes. However, the deregulation profiles of these genes are only documented for a few thyroid toxicants in rats and there is a need for more data to establish whether transcriptomics can be used to screen perturbation of thyroid hormone synthesis by chemicals. Several studies were conducted in rats and allowed to establish profiles of transcript changes following exposure to thyroid active or inactive products.

The rat thyroid cell line PC-Cl3 is documented to express major genes involved in the regulation of the synthesis of thyroid hormones, including Thyroperoxydase (Tpo), Sodium-Iodine Symporter (Nis, Slc5a5), thyroglobulin (Tg), TSH receptor (Tshr), and Pendrin (Slc26a4). We demonstrated that exposure of the cells to four thyroid active compouds caused transcript changes.

The alginate embedded cryopreserved rat primary hepatocytes Liverbeads showed the ability to reproduce the induction of transcription of genes coding for the enzymes involved in the catabolism of thyroid hormones that occurs *in vivo* after exposure of several chemicals.

We thus identified the two cellular models described above as serious candidates for the use in *in vitro* early screening tests for the thyroid function disruption.

Role of antitumoral immunity in the treatment of peritoneal carcinomatosis

Barbara Zunino, Ophelie Meynet, Elodie Villa, Sebastien Pommier, Camila Rubio Patino, Laura Mondragon, Els Verhoyen, Aurore Cornille, Johanna Chiche, Jean-Marc Bereder, Daniel Benchimol, Michel Carles, Jean-Ehrland Ricci*

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

Peritoneal carcinomatosis is a disease of digestive–tract cancer with a median survival of about 2 years. A new and very interesting therapeutic approach is to combine cytoreductive surgery with Hyperthermic IntraPeritoneal Chemotherapy (HIPEC) leading to a median survival of 5 years.

The aim of our work is to uncover how HIPEC could enhance the patient's survival?

We speculated that the immune system could participate in the protective effect brought by HIPEC in patients. Using a murine colon carcinoma cell line (CT26), we generated an in vitro model of HIPEC allowing us to investigate the role played by the chemotherapy alone or in combination with hyperthermia (mimicking HIPEC). We established ex vivo that HIPEC could activate T cells. In addition, when mice were immunized with HIPEC-treated tumor cells they could be protected from a subsequent challenge using the same tumor in viable form (anti-tumor vaccination assay). Then we showed the involvement of heat shock proteins 90 (Hsp90) in the observed effect using in vitro, ex vivo and clinical samples, Indeed, when Hsp90 was blocked using a specific inhibitor (17AAG), we lost the protection induced by the HIPEC-treated cells, therefore underling the role of hsp90 in this HIPEC-dependent induction of anti-cancer immune response.

Taken together, our results raise a new concept indicating that HIPEC could protect patients, at least in part, by mediating an efficient immune response against the cancer cells. In addition, we demonstrated that this effect was mediated by hsp90 release. Our work also suggests that blood levels of hsp90 could be used to predict the overall patient response to the treatment.

The Telomeric Protein TRF2 is an Angiogenic Target of WT1 by Binding and Activating the PDFGRβ Promoter

Mounir El Mai, Kay-Dietrich Wagner, Jean-François Michiels, Damien Ambrosetti, Arnaud Borderie, Sandrine Destree, Valérie Renault, Nadir Djerbi, Marie-Josèphe Giraud-Panis, Eric Gilson, and Nicole Wagner*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Telomeric repeat-binding factor 2 (TRF2), which plays a central role in telomere capping, is also frequently increased in human tumors. We reveal here that TRF2 is expressed in the vasculature of most human cancer types but could not be detected in the vessels of healthy adjacent tissues. In vitro TRF2 over-expression in Human umbilical vein endothelial cells (HUVEC) resulted in an increased proliferation and migration, while silencing of TRF2 led to the opposite results. No changes in apoptosis could be observed. It is worth noting that modulation of TRF2 does not change the level of DNA damage response and that the stimulation of endothelial cells is ATM-independent. The angiogenic effects of TRF2 are then uncoupled from its function in telomere capping. Since the transcription factor WT1 (Wilm's tumour suppressor 1) is highly expressed in human tumour vessels in vivo and mediates angiogenic properties of endothelial cells, we investigated whether TRF2 expression could be regulated by WT1. Indeed, WT1 binds the TRF2 promoter and activates TRF2 transcription. Finally, we found that TRF2 binds and transactivates the promoter of the angiogenic tyrosine kinase PDGFRβ. These findings reveal an unexpected role of TRF2 in neoangiogenesis and delineate a distinct function of TRF2 as a transcriptional regulator.

Beta-cell regeneration induction using a chemical compound

Ben Othman Nouha, Gjernes Elisabet, Courtney Monica, Faurite Biljana, Vieira Andhira, Avolio Fabio, Druelle Noemie, Collombat Patrick*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Background and aims: Type 1 diabetes arises as a result of a cell-mediated autoimmune destruction of insulin-producing pancreatic beta-cells. One avenue of research for a potential therapeutic strategy is cell replacement therapy using cell differentiation/reprogramming to turn different cells sources into beta-cells by mimicking embryonic development.

Using the mouse as a model, we previously showed that embryonic pancreatic glucagon-producing cells can regenerate and convert into insulin-producing beta-like cells through the constitutive/ectopic expression of a single gene, Pax4 (a gene involved in the embryonic specification toward the beta-cell fate). More recently, we demonstrated that the misexpression of Pax4 in glucagon-expressing cells age-independently induces their conversion into beta-like cells.

The regenerative capacity of glucagon-producing cells and their potential for conversion into beta-like cells by the simple ectopic expression of Pax4 are of interest in the context of type 1 diabetes research. However, this transgenic approach would be an impractical approach in humans. We therefore initiated a number of screens aiming to discover small molecules/chemical compounds mimicking the effects of the ectopic expression of Pax4.

Methods: The compound X was found to induce the conversion of a majority of alpha-cells into beta-like cells in vitro.

In vivo tests were then initiated using Glu-Cre::Rosa26-lox-beta-gal mice. These mice, where glucagon-expressing cells are irreversibly marked, were treated with a high dose of streptozotocin and then daily injected (or not) with the compound X once they were hyperglycemic.

Results: In the animals treated with the compound X (isolated 40 days post-streptozotocin injection), islets appeared regenerated and further immunohistochemical analyses using antibodies raised against insulin and beta-galactosidase outlined a majority of cells positive for insulin and beta-galactosidase.

Conclusion: Using lineage tracing experiments, we demonstrated that, upon compound X addition, beta-like cells are regenerated following streptozotocin treatment, these deriving from cells that expressed the glucagon hormone.

Role of the XPC protein in the development of cutaneous cancers

S. Rouanet, Y. Gache, F. Larcher, M. Del Rio, T. Magnaldo*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Somatic stem cells are the origin of tissue renewal, healing in case of injury, and probably cancer development following exposure to genotoxic agents. Ultraviolet radiations (UVs) from sunlight are the major etiological factor of skin cancers. Exposure to UVs, notably UVB, generates mutagenic DNA lesions at bipyrimidine sequences; these lesions are repaired by the nucleotide excision repair (NER) mechanism. Xeroderma pigmentosum (XP-C) is a rare genetic disorder due to a defect in NER. XP patients are highly photosensitive and prone to cutaneous cancers in photo-exposed skin. XP skin cells constitute a highly sensitive model to analyze the mechanisms involved in cancer development.

We have regenerated XP-C skin onto SCID mice and first studied the effects of acute UVB irradiation. Under these circumstances, invasive epidermal structures developed after 3 months and exhibited substantial alterations of differentiation as observed in human carcinomas. Conversely, skin regenerated from genetically corrected XP-C keratinocytes presented a normal histology and proper features of differentiation and stratification. To study the effects of chronic UVB, XP-C keratinocytes were submitted to low doses irradiations. The clonogenic potential of chronically irradiated keratinocytes was dramatically increased compared to non-irradiated controls. Sequential irradiations also allowed us to isolate atypical keratinocytes clones that genome structure and expression is under study. Skin regeneration from these “UV-primed” cells will reveal their neoplastic potential. For the first time, these studies will allow us to decipher the sequence of genetic alterations and their consequences upon acute and chronic UV carcinogenesis in the human.

Key Role Played By SOX11 During Nephrogenesis

Haroun Dhib, Yasmine Neirijnck, Antoine Reginensi, Filippo Massa, Andreas Schedl*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

For reasons of confidentiality, this abstract may not be published.

VapBC-type toxin-antitoxin modules of *Sinorhizobium meliloti* influence bacteroid survival and nitrogen fixation in symbiosis with the host plant *Medicago*

Lipuma Justine, Aurélie Kiers, Isabelle Garcia and Laurence Dupont*

**ISA - Institut Sophia Agrobiotech, Sophia-Antipolis - UMR INRA 1355 - CNRS 7254 - UNS*

The symbiotic interaction between the bacterium *Sinorhizobium meliloti* and the legume *Medicago truncatula* results in the development of new root organs, the nodules, where the differentiated bacteria (called bacteroids) reduce atmospheric N2 to ammonia. Few weeks after symbiosis establishment, nodules present a premature senescence, which begins with death of bacteroids. Since VapBC-type Toxin-Antitoxin (TA) systems of animal pathogenic bacteria have been shown to influence the survival in eukaryotic hosts, we examined the possible role of *S. meliloti* VapBC modules in bacteroid viability and nodule senescence. The VapBC systems represent the most abundant class of TA in the genome of Bacteria and Archea. These systems consist of a toxin (VapC) neutralized by the associated-antitoxin (VapB) that form a TA complex which negatively regulates the transcription of the vapBC operon. Upon stress, the unstable antitoxin is degraded and the stable toxin is free to act as a regulator of the translation due to its specific RNase activity.

During the two first years of my thesis, we have examined the consequence of a mutation in the toxin component of two different vapBC modules of *S. meliloti,* on the symbiotic efficiency with *Medicago* sp. These results will be presented and the role of the TA systems in the intracellular lifestyle of the endosymbiotic bacteria will be discussed.

Briefly, we showed that, during the symbiotic interaction with *M. sativa* (alfalfa), the mutant deficient in the VapC5 toxin of the VapBC5 module, leads to a higher nitrogen-fixing activity, plant yield increase and a delayed nodule senescent phenotype. Inactivation of this toxin improves symbiotic efficiency.

The symbiotic phenotype of the second toxin mutant (vapC7) in interaction with *M. truncatula*, showed an opposite result. Indeed, this mutant was strongly affected in symbiotic capacity: aberrant nodules were developed; nitrogen fixation was nearly abolished resulting in altered plant yield. Nodule ultrastructure analysis, coupled to flux cytometry, demonstrated that bacteroid differentiation was followed by a rapid bacterial death leading to early senescence of the nodules. These results show that this VapBC module is essential for bacteroid viability and symbiotic interaction.

Deciphering the role of Rab8 in Hh trafficking

Tanvi Gore, Tamas Matusek, Pascal Therond*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Hedgehog (Hh) is a highly conserved and secreted morphogen which plays an essential role in several processes like embryonic development, stem cell maintenance and tissue homeostasis. Hh protein acts at both short and long ranges within the receiving cells directing cell fate and development. Hh undergoes post-translational modifications and becomes dually lipidated making Hh hydrophobic and membrane associated. We asked how the secretion, extraction and transport of Hh protein are regulated at the place of its production. Therefore, we designed and performed a genome-wide RNAi screen in Drosophila to identify new regulators of Hh secretion and obtained Rab8, a novel component of the Hh secretory pathway.

Rab8 is a monomeric GTPase belonging to the Rab8-Rab10 family. Its role in intracellular transport is debated over; it was originally described to regulate the protein delivery from the trans-Golgi network to the basolateral side in MDCK cells. But conversely, in mice, Rab8 knockout causes defects in the delivery of proteins to the apical side in the intestinal cells and causes them to accumulate in vacuoles.

According to our current model there are at least two pools of secreted Hh. The apical pool is needed for the long range target gene activation and basolateral pool for the short range target gene activation. It is not clear how Hh is transported apico-basally. We found that partial loss of Rab8 in Hh producing cells impairs Hh apicobasal distribution. Hh is seen to be excluded from the apical domain suggesting defects in apical Hh secretion. Moreover, loss of Rab8 function in the Hh producing cells reduces Decapantaplegic (dpp) activity, one of the long range Hh targets and leads to Hh hypomorphic loss-of-function phenotype on the adult Drosophila wing. This suggests that interfering with Rab8 function in the Hh producing cells impairs Hh secretion thus reducing the Hh gradient activity. We have isolated protein null Rab8 mutants and are testing whether Rab8 regulates apical or basal transport of Hh or whether it is involved in Hh recycling. We hope that deeper understanding Rab8 function by using a true loss-of function analysis combined with cell biology approaches will help to further dissect the regulation of Hh transport and secretion.

Targeting mitochondria through modulation of eIF5A hypusination protects from anoxia-induced cell death

Nicolas Melis, Christophe Duranton, Isabelle Rubera, Marc Cougnon, Baharia Mograbi, Didier Pisani and Michel Tauc*

**LP2M - Laboratoire de PhysioMédecine Moléculaire, Nice - CNRS UMR 7370 - UNS*

eIF5A, for eukaryotic initiation factor 5 A, is a highly conserved protein throughout evolution. Firstly described as a translation initiation factor, it became more noteworthy for its unique post-translational activation through hypusination. This modification, a 4-aminobutyl moiety from spermidine transferred onto a lysyl residue, is sequentially catalyzed by two enzymatic steps involving respectively the deoxyhypusine synthase (DHPS) and the deoxyhypusine hydroxylase (DOHH). We took advantage of this unique characteristic and use the highly specific DHPS competitive inhibitor GC7 (N-guanyl-1,7-diaminoheptane) to demonstrate an unsuspected link between eIF5A hypusination, mitochondria activity and the cellular resistance to anoxia.

To investigate the involvement of this pathway in the resistance to low oxygen level, renal proximal tubular cells (PCT) were exposed to anoxia (<0.1% O2, 24 h). GC7 pre-treatment (30µM) or RNA silencing-mediated inhibition of DHPS or DOHH largely protected from the anoxia-induced cell death. This tolerance to anoxia is paralleled by a marked increase in glucose consumption and lactate production reflecting a reversible metabolic shift from aerobic OXPHOS to anaerobic glycolysis, preserving the cellular energetic status. We also studied the effect of GC7 on mitochondrial status and showed that GC7 induced a reversible “mitochondrial silencing” characterized by a decrease in mitochondrial potential (ΔΨm) and a drastic mitochondrial structure remodelling associated to a down-regulation of respiratory chain complexes expression. The resulting effect is a decrease in O2 requirements associated with a reduction of the deleterious reactive oxygen species produced during anoxia.

Thus, targeting mitochondria through modulation of eIF5A hypusination pathway may offer an innovative therapeutic strategy for ischaemic human diseases —e.g., stroke or myocardial infarction—or organ transplantation.

Fractalkine and experimental bone metastases of lung cancer origin: a preclinical study of immunotherapy

Emilie Goguet-Surmenian, Rasha Al-Salhanee, Marie-Ange Millet, Damien Ambrosetti, Jean-François Michiels, Pierre Alemanno, Annie Schmid-Alliana, Heidy Schmid-Antomarchi*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Bone metastases derived from cancers at advanced stages, such as breast and lung cancers, are devastating and incurable. They are associated with the development of skeletal-related events, such as pathologic fractures or bone pain, through bone metabolism disruption and the development of a « vicious cycle », linking tumor proliferation and bone resorption. Despite the therapeutics progresses accomplished lately, bone metastases are still associated with a poor prognosis for the patient, due to the fact that, with time, the disease progresses to a phase in which standard therapy fails to control the malignancy, and then progresses further to a highly chemotherapy resistant state. Therapeutic strategies aimed at preventing or delaying their development, and allowing to limit the potential side effects of systemically-delivered drugs, are therefore considered a major public health issue.

Fractalkine (CX3CL1) is a chemokine existing under both a secreted (soluble) and a membrane-bound form. The soluble form was described as a good candidate to stimulate a strong antitumor immune response in various forms of cancers. But on the other hand, it could be involved in the homing of tumor cells expressing its receptor (CX3CR1) to bone sites, because of its expression/secretion by osteoblasts. Fractalkine could also be involved in the bone metastatic « vicious cycle », through its involvement in the osteoclastic differentiation (CX3CR1 expression on osteoclast precursors). Could we then elicit an efficient antitumor immune response and disrupt the vicious cycle established between the tumor cells and the osteoclasts? And how best to achieve this result: should we block or promote the CX3CL1/CX3CR1 axis?

We developed a syngeneic murine model of experimental bone metastases of lung origin and compared the extent of tumor development between LL2 lung cancer cells expressing the membrane-bound, the soluble fractalkine or none (control).

The membrane-bound form promoted tumor growth in a bone location, increasing the bone resorption and the ability of the tumor cells to invade adjacent tissues (muscles) when compared to control tumor cells. The soluble form significantly reduced the tumor size, the bone resorption and the invasiveness of the tumor cells when compared to the membrane-bound form and the control tumor cells. Key genes in tumor aggressiveness, osteoclastic differentiation and bone formation were differentially expressed when comparing the three conditions. We showed a significant difference in the contribution of the infiltrating leukocytes to the anti-tumor activity of soluble fractalkine.

Soluble fractalkine expression by LL2 tumor cells drastically reduced the tumor development and the extent of bone resorption and therefore could represent a promising tool in the therapeutic arsenal against bone metastasis.

Signalling pathways regulating the generation of brown and white adipocyte progenitors during human embryonic development in vitro

Hafner Anne-Laure, Mohsen-Kanson Tala, Villageois Phi, Wdziekonski Brigitte, Takashima Yasuhiro, Smith Austin, Dani Christian*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Brown and white adipose tissues coexist in mammals. Both are involved in the energy balance but have opposite functions: whereas white adipose tissue (WAT) is mainly involved in energy storage, brown adipose tissue (BAT) is specialized in energy expenditure. Although BAT is mainly present in newborns and decreases with aging in humans, metabolically active BAT exists in adults and contributes to the energy balance. Therefore, identification of pathways regulating brown and white adipocyte development in humans is of fundamental importance and could ultimately be clinically applicable. However, nothing is known on the events regulating adipocyte progenitor (AP) generation in humans due to the lack of cellular model. The potential of pluripotent stem cells (induced pluripotent stem cells (iPSCs), and embryonic stem cells), to generate adipocytes has dramatically enhanced the prospects for investigating the earliest steps of adipogenesis during human embryonic development.

We have recently reported that brown and white APs generation is regulated by retinoic acid and TGFb pathways during in vitro development of human pluripotent stem cells 1. APs of both phenotypes were derived from hiPScs cultures and their molecular characterization revealed that Pax3 marked brown-like APs. Functional experiments indicated that Pax3 transcription factor was a critical player of human AP fate as its ectopic expression led to reprogram white APs into brown-like APs. The molecular mechanisms mediating Pax3 effects are under investigation.

hiPSc differentiated cultures are composed of a complex mix of different cell types, as expected for the development of pluripotent stem cells. Interestingly, we and others observed that APs displayed a weak differentiation potential when isolated from other cell types, strongly suggesting that hiPS microenvironment was required to promote adipogenesis. To test this hypothesis, hiPSc-APs were induced to undergo differentiation in the presence of hiPS-conditioned media. Preliminary experiments showed that soluble factors, which dramatically enhance the potential of APs to undergo differentiation, were secreted during hiPSc development.

The findings of the present study could lead to characterize the human AP niche and to new anti-obesity therapies based on the recruitment of APs.

Role of the TREK2 background potassium channels in nociception

Christin M., Pereira V., Devilliers M., Legha W., Alloui A., Lazdunski M., Busserolles J., Eschalier A., Noël J.*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

The perception of noxious stimuli is essential to an organism survival as it allows the appropriate avoidance response to potentially harmful situations. This detection occurs at the peripheral terminals of specialized sensory neurons - nociceptors. These neurons of small diameter transduce stimuli of a thermal, mechanical or chemical nature into action potentials and transmit this information to the spinal-cord. The stimulation of nociceptors at the periphery by the different stimuli relies on the expression profile of specific ion channel transducers at the plasma membrane of the axon terminals. The nociceptive system is characterized by a high degree of plasticity which is exacerbated in patho-physiological conditions.

We study the role in nociception of background K+ channels with two-pore domains (K2p) belonging to the TREK channels subfamily. The K2p channels generate background K+ currents that play a major role in neuronal excitability and cell firing. The TREK channels subfamily is composed of TREK1, TREK2 and TRAAK. They are mechano- and thermo-activated channels that have been involved in anesthesia, depression and neuroprotection. We have previously demonstrated the role of TREK1 and TRAAK channels in polymodal pain perception (Alloui et al., 2006; Noel et al., 2009). They are involved in mechanical pain as well as in heat and cold perception. They prevent nociceptive fibers from firing at moderate temperature by opposing the depolarization resulting from the gating of excitatory channels by temperature. Whilst TREK2 is the major background K+ current in dorsal root ganglion neurons and shares many regulations and functional properties with TREK1 and TRAAK, its role in nociception still remains unknown. The aim of our work is to investigate the role of TREK2 in nociception in physiological and patho-physiological conditions using knock-out mice. We assessed the impact of this channel on nociceptors with complementary *in vitro*, *ex vivo* (nerve-skin recordings), and pain behavior tests. We show that each member of the TREK channels subfamily contributes to thermal perception in different temperature ranges and that they are involved in neuropathic and inflammatory pain.

RÉSUMÉS DES POSTERS

Poster n°1

Analysis of skin cancer development with the xeroderma pigmentosum model

Y. Gache, S. Al-Qaraghuli, S. Rouanet, S. Scarzello, C. Girard, M. Ohanna, C. Bertolotto, R. Ballotti, T. Magnaldo*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Our objectives are to study the mechanisms implicated in the development of human epithelial cancers among which skin cancers (squamous cell carcinoma, SCC, and malignant melanoma, MM) account for the most aggressive neoplasms. The ethiology of those cancers is clearly linked to exposure to genotoxic stresses, notably ultraviolet radiations (UVR). In addition, increasing evidence supports the idea that activation of stromal cells, notably dermal fibroblasts, in turn, promotes tumor cells aggressiveness and invasive capacities. Xeroderma pigmentosum (XP-C) is a rare genetic disorder characterized by a severe predisposition to aggressive skin cancers following minimal exposure to UVR. XP-C cells are deficient in the nucleotide excision repair (NER) of DNA lesions introduced at bipyrimidine sequences following UVR. Beside a tremendous quantitative increase in skin cancer development, cancers in XP patients are particularly aggressive leading to compromised life expectancy. On these clinical bases, we hypothesized that XP-C fibroblasts could further promote the aggressiveness of patients cancers. We have analyzed the capacity of XP-C fibroblasts to promote migration and invasion of melanoma and carcinoma cells. Our results show that the secretome of XP-C fibroblasts elicited migration and invasion of SCC12/SCC13 cells, and of the non-tumorigenic Mel501 melanoma cells in vitro. Treatment of Mel501 cells in culture supernatant of XP-C fibroblasts provoked tumorigenesis in mice. Invasion and tumor formation were abolished in genetically corrected XP-C fibroblasts. Screening of molecules secreted by XP-C fibroblasts identified Hepatocyte Growth Factor/Scatter Factor as a potential pro-invasive/tumorigenic factor. Culture supernatants from XP-C fibroblasts activated the c-Met signaling pathways in SCC and Mel501 cells. Our studies demonstrate an important role for XP-C fibroblasts in tumor progression and identify factors responsible for the formation of a permissive microenvironment.

Poster n°2

TRF2 overexpression increases oral squamous cell carcinomas severity: a potential role for the microenvironnement

Y. Benhamou, V. Picco, G. Pages*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Oral cancer kills every year 5000 people in France with an incidence of 14000 new cases. The oral squamous cell carcinoma (OSCC) subtype represents 90% of all oral cancers and is defined by an abnormal proliferation of epithelial cells composing oral mucosa.

Dental surgeon is regularly consulted for diagnosing and treating alcohol-tobacco intoxicated patients, with mucosal lesions.

Recent improvements in understanding biology of cancer resulted in identifying cancer potential targets and developing targeted chemotherapies. Although these treatments show better efficacy and less side effects, some tumors are resistant to these treatments.

TRF2 (Telomeric Repeat-binding Factor 2) , a protein associated with distal ends of human chromosomes, is overexpressed in various cancers as like as the EGF receptor which overexpression is targeted by therapies such cetuximab and erlotinib. Recent studies have proven that TRF2 overexpression was associated with increasing of cancer incidence and shorter survival in animals, but none were conducted in humans.

Our team has shown in a cohort of 62 patients a strong association between a high level of TRF2 protein expression in immunohistochemistry and a decrease in overall survival

In order to unravel which mechanisms are underlying tumor agressiveness when TRF2 is overexpressed, OSCC cell lines were studied in our lab, modulating levels of TRF2 expression. ShRNA and dominant negatives approaches were conducted in order to respectively lower TRF2 expression or disrupt its function. We also performed TRF2 overexpression in these cell lines. Signaling pathways, cell metabolism and cellular proliferation, treatment responses and invasive capacities with various TRF2 levels were analyzed and we observed no intrinsic difference between TRF2 modulated and control cells in any of these conditions. These results suggest that the interaction between tumor cells and their microenvironment is responsible for the higher aggressiveness of the tumors expressing a high level of TRF2.

Understanding TRF2 specific role in OSCC cells and what leads to pejorative evolution could change patient care. After a routine oral biopsy, an analysis in immunohistochemistry of TRF2 level -as like in HER2 breast cancer- would allow a better targeting of therapies. TRF2 would become a new independant marker for survival and treatment response, facilitating management of patients with such tumors.

Poster n°3

The Arf6 Exchange Factor EFA6 and endophilin directly interact at the plasma membrane to control clathrin-mediated endocytosis

Boulakirba Sonia, Macia Eric, Partisani Mariagrazia, Lacas-Gervais Sandra, Brau Frédéric, Luton Frédéric, Franco Michel*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

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, w e 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.

Poster n°4

The L-type amino acid transporter 1 (LAT1) component of the CD98/LAT1 complex is essential for tumor growth

Y. Cormerais, R. LeFloch, J. Durivault, L. R de la Ballina, S. Giulano, C. Féral, M. Palacin, N.M. Mazure and J. Pouysségur*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

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 teratocarcinomas formation. This pro-tumoral action was reported to be due more to the key role of integrins via CD98 signalling than the transporter LAT1 itself despite the fact that CD98 knock-out mice have reduced expression of LAT1.

Here we investigated the pro-tumoral role of LAT1. We knocked-out LAT1 in the colon adenocarcinoma cell line LS174T using Zinc Finger Nucleases (ZFN). The corresponding surface expression of CD98 was decreased by more than 70 % in two independent LAT1-null clones. We then designed an experiment to fully re-express endogenous CD98 at the cell surface in LAT1-KO cell lines. For this we generated in vitro a series of point mutations in LAT1 cDNA able to abolish the transport activity. This transporter-dead LAT1 cDNA expressed in the two ZFN-mediated LAT1-KO cell lines was able to fully re-express CD98 at the plasma membrane,

LAT1-null, CD98low cells display a 2- to 3-fold reduction in growth rate in normal DME medium, a constitutive activation of the eIF2/ATF4 amino acid stress response pathway and a sharp reduction of mTORC1 activity. Exposing these cells to a 3-fold reduced concentration of amino acids fully abolished mTORC1 activity and clonal growth while control cells were able to sustain proliferation and mTORC1 activity.

Interestingly CD98-reconstituted cells: LAT1-null, CD98high cells display the same in vitro proliferation defect phenotype as described for the LAT1-null, CD98low cells, indicating no growth benefit for the re-expression of CD98.

These findings highlight the specific pro-tumoral contribution of LAT1 in the heterodimeric CD98/LAT1 complex. Future in vivo experiments will address the respective role of CD98 and LAT1 via comparing colon adenocarcinoma with the highly migratory glioblastoma U87 tumor cells.

Poster n°5

Childhood-onset schizophrenia: epidemiological, social cognition and genetic exploration.

E. Dor, F. Askenazy*

**CHU - Centre Hospitalier Universitaire, Nice*

Childhood-onset schizophrenia (COS) is a rare but severe psychiatric disorder with important individual, family and societal consequences. COS is a disease whose diagnosis is complex. Its boundaries are ill-defined because the disorder is inherently pervasive and manifests itself in very different ways from one child to another. Plus, it is part of a set of serious disorders or \"childhood psychoses\" which themselves overlap and therefore lack clinical specificity. Unlike the majority of psychopathological disorders of childhood and adolescence, COS is defined and diagnosed using the same criteria as those used in adults. There are several forms of schizophrenia based on the presence of one symptom or another or based on age criteria. This is when the definitions appear for very-early-onset schizophrenia (VEOS) occurring before the age of 12 and early-onset-schizophrenia (EOS) occurring between 12 and 18 years.
In the field of child psychiatry, links between autism spectrum disorders (ASD) and VEOS are not yet well known. They are neuro-developmentally serious diseases with very severe prognosis and consequences. Additionally these disorders are considered to be resistant to antipsychotic treatment.
Some literature data are beginning to report a significant risk of progression of certain forms of autism spectrum disorder (ASD) to VEOS. Currently, there is no prospective study to determine the incidence of pre-pubertal patients with an additional diagnosis of ASD and schizophrenia, nor the risk of conversion of ASD patients to a VEOS., VEOS (before age 12) remains a poorly understood clinical entity because of its low incidence (1 0/0 to 30/0) and the difficulty in carrying the clinical diagnosis (positive dimension and disorganization are complex to evaluate). Literature in child psychiatry and preliminary work of our teams show a clinical and neuro-cognitive cross between these two spectra (62.5% co-morbidity TED / STP in our preliminary cohort VEOS).
Neuropsychological disorders in schizophrenia and ASD children, especially in the area of executive functioning, social cognition, language and communication, are remarkably similar. Disorders of social cognition are better described (noted?) in autistic patients than in schizophrenic patients, particularly with regard to the field of theory of mind, recognition of facial emotions, perception of holders index of social significance.
For the VEOS a growing number of arguments consider the disease as a neuro-developmental disorder associated with genetic and / or environmental vulnerability.
Methods: It is in this context of research we developped:
(1) an epidemiological study to assess the prevalence of schizophrenia in the child population supported in medico-social and health structures in PACA.
(2) a clinical study of neurocognitive profile of 20 children with EOS compared with neurocognitive profile of 20 children with ASD and 20 healthy subjects, particularly in social cognition (theory of mind, attribution of intent and perception of affects).
(3) a genetic study of children and their families with EOS and ASD
Results: First study: 432 children selected and 299 included. 29 have a SCZ diagnosis (9.7%). Second study: we found differences between the social cognition of children with EOS versus children with ASD and healthy subjects. Third study: ongoing, inclusion phase.

Poster n°6

Azacitidine-resistant MDS cells exhibit defective-chaperone-mediated autophagy and increased lysosomal activity

Alix Dubois, Clémence Ginet, Wejdene El Manaa, Amine Hamouda, Frédéric Luciano, Sandrine Marchetti, Sandrine Obba, Diogo Gonçalves, Anja Pfeifer, Arnaud Jacquel, Pascal Colosetti, Guillaume Robert [$] and Patrick Auberger [$]*

[$] *Equal contribution*

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

Azacitidine (Vidaza®) is the leading treatment for patients suffering high-risk myelodysplastic syndrome (MDS). However, a majority of patients appears primary refractory or relapses early under treatment. These patients exhibit a significantly reduced life expectancy compared to the sensitive one. Therefore, identification of predictive factors for AZA resistance could be of the greatest interest to propose rapidly alternative therapeutic strategies for non-responsive patients.

To investigate the putative mechanisms of resistance to Azacitidine, we generated Azacitidine-resistant cells (AZA-R) from the SKM1 myeloid cell line and showed that these cells exhibited a profound defect in apoptosis induction upon Azacitidine treatment. Accumulation of intra-cytoplasmic vesicles was also observed in AZA-R cells that reflected increased autophagy. Importantly, autophagy inhibitors were found to trigger AZA-R but not AZA-S cell death. AZA-R cells exhibited a global loss of protein expression and a defective chaperone-mediated autophagy but a net increase in lysosomal mass compared to their AZA-sensitive counterparts. In addition, AZA-R cells showed high dependency on their lysosomal activity for growth and survival, since lysosomotropic agents efficiently eliminated AZA-R but not AZA-S cells. We are currently trying to confirm these findings using MDS cells derived from patients.

In conclusion, our work has allowed a better understanding of Azacitidine resistance and paved the way for targeting autophagy and lysosomal activity in AZA-resistant MDS patients.

Poster n°7

The Syk tyrosine kinase suppresses motility and metastasis of melanoma cells by regulating β1 integrin-based adhesion

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

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

The progression of tumors to the metastatic disease involves the loss of metastatic suppressor functions. The propensity to metastasize is particularly high in melanoma, a potentially fatal form of skin cancers. Thus, aberrant cell migration is a key feature of melanoma progression, and is required for metastasis. Consequently, genetic and epigenetic changes that dysregulate cell motility, are likely to be important for the pathogenesis of melanoma. The spleen tyrosine kinase (Syk) is a cytoplasmic tyrosine kinase that has been implicated in tumor suppression of many cancers such as breast and pancreatic cancers and melanoma. In melanoma Syk is frequently downregulated by epigenetic silencing and its loss has been associated with senescence escape during melanomagenesis, but whether it also regulates tumor cell motility and metastasis remains poorly understood. In this work we further investigated its tumor suppressive activity by using gain- and loss-of-function approaches to analyze the effects of Syk on the motility of human and murine melanoma cells. We found that expression of Syk (by adenoviral delivery) or knockdown of Syk (by siRNA) results in decreased or increased migration and invasion of melanoma cells, respectively. In vivo monitoring of experimental metastasis revealed that reexpression of Syk in Syk-deficient cells decreases their ability to form lung metastases. 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 tyrosine kinase activity. Mechanistically, we found that silencing of Syk upregulated integrin β1 activity and enhanced melanoma cell adhesion to fibronectin and collagen two ligands that support integrin β1-mediated cell attachment. Silencing of Syk also resulted in increased focal adhesion numbers and FAK-dependent signaling. Importantly, interfering with β1 integrin function using antibody-mediated blockade prevented enhanced adhesion elicited by Syk knockdown. Together 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 melanoma development and acquisition of metastatic potential.

Poster n°8

Let-7i-5p and miR-125b-3p as potential regulators of the conversion of white into brown adipocytes

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

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Brown Adipose Tissue (BAT) has long been thought to be absent or very scarce in human adults. The recent discovery of thermogenic BAT in human adults opened the field for innovative strategies to combat overweight/obesity and associated diseases such as type 2 diabetes. This energy-dissipating function of BAT is responsible for adaptive thermogenesis in response to cold stimulation. In this context, adipocytes can be converted, within white adipose tissue, into multilocular adipocytes expressing UCP1, named “inducible brown”, “brite” or “beige” adipocytes. We take advantage of our human cellular model (hMADS) to identify and/or validate critical factors involved in the induction of a thermogenic program within adipocytes. Among these factors, we evaluate the role of microRNAs as novel regulators of brown/”brite” adipocyte differentiation and conversion. We carried out a miRNA microarray and identified four miRNAs that were down-regulated upon a prolonged rosiglitazone treatment which leads to britening of hMADS cells. We focused our interest on let-7i-5p and miR-125b-3p. We validated their down regulation in hMADS cells and in differentiated human adipocytes derived from primary cultures of adult donors. Finally the expression of let-7i and miR-125b-3p was also found down-regulated in adipose tissues of cold-exposed and β3-adrenergic receptor agonist (CL316 243) treated mice. Functional studies in hMADS cells demonstrated that over-expression of let-7i results in inhibition of mRNA as well protein UCP1 expression and oxygen consumption. Over-expression of miR-125b-3p decreases the basal oxygen consumption and maximal mitochondrial respiration rate. Injection of the miRNA directly in the BAT and sub-cutaneous adipose tissue of mice treated with CL316 243 leads to a decrease of mRNA UCP1 expression in this tissue. Altogether, our data show that let-7i-5p and miR-125b-3p represent important miRNAs candidates involved in the formation of brite adipocytes and potential target to prevent and/or cure overweight and obesity.

Poster n°9

Role of primary cilium during adipocyte differentiation

Forcioli-Conti Nicolas, Dani Christian, Peraldi Pascal*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Obesity is characterized by an increase in the size and number of adipocytes. Thus, a complete understanding of adipocyte differentiation is a paramount importance. Here, we have studied the involvement of the primary cilium during adipocyte differentiation. The primary cilium is an organelle, present in most of the cells in mammals, thought to act as a « signaling antenna ». Its axoneme is composed of acetylated tubulin. The primary cilium is a post-mitotic organelle which is disassembled by the tubulin deacetylase HDAC6 during mitosis. This organelle is also necessary for hedgehog signaling. This signaling pathway has been shown to inhibit adipocyte maturation of mesenchymal stem cell. Moreover ciliopathies, such as the Bardet Biedl syndrome are associated with obesity, suggesting an important role for the primary cilium during adipocyte differentiation.

We have observed that the primary cilium disappears during adipocyte differentiation. Together, the primary cilium appears as an interesting candidate to understand adipocyte differentiation in normal and pathological conditions.

In this study, we try to understand (i) why and how does the primary cilium disappear during adipocyte differentiation and (ii) what is the function of this loss.

We observe that, (i) HDAC6 increases during adipocyte differentiation. Moreover, the pharmacological inhibition by tubacin of HDAC6 maintains primary cilia and is a potent inhibitor of adipocyte differentiation.

(ii) AMPK, known to interfere with adipocyte differentiation, seems to be regulated by the primary cilia in mesenchymal stem cells. LKB1, responsible of AMPK activation is located in the cilium. The activated AMPK, and its substrate ACC, are localized at the cilium base. Furthermore, stimulation of AMPK by metformin leads to its relocation around the cilium. In this case, AMPK activation inhibits adipocyte differentiation and maintains primary cilia. Finally, the AMPK activation leads to an accumulation of acetylated tubulin. This observation suggests an important connection between HDAC6 and AMPK pathway.

These data support the hypothesis of a strong connection between primary cilia and adipocyte differentiation. Moreover, the primary cilium seems to act as a control cassette for AMPK signaling pathway. This cassette is capable to modulate acetylation or deacetylation level of α acetylated tubulin, and thus would have an impact on primary cilium dynamics, which would be a complete new role for AMPK signaling pathway. This opens interesting new research field for the understanding of adipocyte differentiation and mesenchymal stem cells metabolism.

Poster n°10

Structure-function of oomycete biofilm and plant disease

Marie Larousse, Catherine Mura, Eric Galiana*

**ISA - Institut Sophia Agrobiotech, Sophia-Antipolis - UMR INRA 1355 - CNRS 7254 - UNS*

The overall interactions between a plant pathogen and the host surface-resident microbiota are critical to disease outcome. They shape the distribution, the density and the genetic diversity of inocula. It is of importance for basic and applied ecology to define individual and communal incidences of these interactions which in most cases remain to be specified. To get insights on functional biodiversity, we rely on the analysis of interactions between the rhizospheric microbial community of Solanum lycopersicum and a polyphagous pathogen, the oomycete Phytophthora parasitica colonizing roots through biofilm formation. For that, we deploy a methodology in three steps by coupling metagenomic and microbiology approaches: (I) to specify the structure of two microbial communities using sequencing of 16S and 18S rRNA genes: a first one delineates the diversity in the rhizosphere, the second one, the microbial diversity associated with the pathogen within the biofilm; (II) to compare the two communities through the definition of identity and richness of each prokaryotes and eukaryotic microorganisms class; (III) to decipher the nature of interactions within the biofilm community which act on the fate of plant disease. To this aim, a collection of prokaryotic (3000) and eukaryotic (3000) isolates is established from the mixed-species biofilm based on their ability to grow in the environment of the oomycete. The microorganisms are screened for interference with disease dissemination, pathogen growth or onset of plant defenses. This approach introduces a new screening method to define the specific microbial environment conducive or detrimental to disease development. This method could be useful for a predictive application in ecological and sustainable agriculture based on use of tools to define the biotic status of a soil with respect to the occurrence of an epidemic. Identification of microbial species acting positively or negatively on pathogen infection outcome could also be expected.

Poster n°11

The chemokine MCP1 mediates inflammation-induced anorexia through its action on hypothalamic MCH neurons

Le Thuc O., Blondeau N., Rostène W., Guyon A., Cazareth J., Heurteaux C., Nahon J.L. and Rovère C.*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

Cytokines and chemokines are secreted by the immune system in response to injuries and/or infections and play an important role in anorexia. Lipopolysaccharide (LPS)-injection induces both pro-inflammatory pathways and weight loss and is used as a model to investigate the central regulation of appetite behavior and body weight by neuroinflammation. The lateral hypothalamic area (LHA) shelters two neuronal populations producing the two major orexigenic peptides: Melanin Concentrating Hormone (MCH) and hypocretin/orexin (ORX). In the present study, we showed by quantitative PCR and immunoassays that central injections of LPS induced a drastic expression of the Monocyte Chemoattractant Protein (MCP-1) mRNA and derived protein in LHA, while MCH and ORX mRNA and proteins were down-regulated. The time frame for this modification suggested that MCP1 overexpression could participate to the LPS-induced down-regulation of both MCH and ORX. Indeed, our results showed that cerebral injections of MCP1 decreased MCH and ORX mRNA and protein levels, similarly to LPS. The MCP1 receptor, CCR2, was also mapped by immunohistochemistry in MCH neurons. We tested therefore whether MCP1 could act directly on these neurons. Secretion and electrophysiology experiments demonstrated that MCP1 application on MCH-neurons in brain tissues decreased their activity. Finally, the use of pharmacological tools and transgenic animals lacking MCP1 and CCR2 confirm the role of MCP1 pathway in LPS-induced anorexia.

This work was supported by the CNRS and FRM.

Poster n°12

Oral intoxication by *Bacillus thuringiensis* generates transient dysplasia in the Drosophila intestine

Rihab Loudhaief, Olivia Benguettat, Alexandra Brun-Barale, Marie-Paule Esposito, Marcel Amichot, and Armel Gallet*

**ISA - Institut Sophia Agrobiotech, Sophia-Antipolis - UMR INRA 1355 - CNRS 7254 - UNS*

Bacillus thuringiensis (*Bt*) is a soil bacterium that is widely used worldwide as bioinsecticides. *Bt* strains produce crystalline toxins, named Cry toxins, among which have been selected in organic farming owing to their lethal properties against specific pests. Because of incentive programs for sustainable development, the use of *Bt* bioinsecticides as an alternative to chemical pesticides will further increase in the next decades. The question now is how far non-target organisms will be potentially impacted by the resulting augmentation of the *Bt* bacterium and its Cry toxins in the environment? To answer this challenge, we are using drosophila (a non-target organism) to study the impacts of the most common *Bt* bioinsecticides on the gut physiology because 1/ the digestive tract is the main entrance for *Bt*-contaminated food, 2/ the gut is the first barrier against exogenous bacteria and compounds and 3/ *Bt* is a mammalian opportunistic pathogen.

The data presented in this poster address the adverse effects caused by the ingestion of low doses (mimicking an environmental contamination) of one of the *Bt* strain widely used in organic farming and forestry: *Bt kurstaki* (*Btk*). We show that the bacterial component of *Btk* does not kill flies, but its ingestion disturbs the physiology of digestive tract, leading to the appearance of a transient dysplasia.

Poster n°13

Role of microRNAs in human airway epithelium differentiation: Characterization of miR-449 as a central player in multiciliogenesis conserved in vertebrates

Olivier Mercey, Benoit Chevalier, Anna Adamiok, Laure-Emmanuelle Zaragosi, Christelle Coraux, Andrea Pasini, Laurent Kodjabachian, Pascal Barbry, Brice Marcet*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

The airway epithelium is the first line of defense which protects the respiratory tract against frequent external aggressions (inhaled toxic particles, pathogens, allergens,…). This epithelium lining the surface of the airways is constituted by basal cells, goblet cells and multiciliated cells (MCCs) which exhibit hundreds of motile cilia. The coordinated beating of these motile cilia is crucial to the mucociliary clearance, essential for airway cleansing. The formation of motile cilia (multiciliogenesis) requires sequentially a cell cycle arrest, followed by a massive multiplication of centrioles which then migrate and dock into a dense apical actin network before axoneme elongation of each cilium. A better understanding of complex mechanisms governing multiciliogenesis could help to develop new regenerative therapeutic strategies for restoring airway epithelium integrity and mucociliary clearance. MicroRNAs (miR or miRNAs) are small non-coding regulatory RNAs implicated in numerous biological processes and more recently associated with several chronic airway diseases. Using two distant models of mucociliary epithelium (in vitro with the human airway epithelium and in vivo with the the Xenopus embryonic epidermis) we have shown that miRNAs of the miR-449 family were specifically expressed in precursors and mature MCCs. Protector oligonucleotides, targeting miR-449 binding site on several targets (Notch pathway and small GTPase R-Ras), indicate that miR-449 promote the cell cycle arrest, control both the amplification of centrioles and the apical actin remodeling, and allow MCC precursors to differentiate. Our findings demonstrate that miR-449 miRNAs are key regulators of vertebrate multiciliogenesis which act as “chefs d'orchestre” by finely controlling several pathways to trigger MCC differentiation.

Poster n°14

Human myogenic progenitors and macrophages inhibit the differentiation of intramuscular adipogenic progenitors to support muscle regeneration

Claudine Moratal, Christian Dani, Claude A. Dechesne*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Human skeletal muscle is a powerful regenerative tissue in response to a variety of injuries. Myogenic progenitors (MPs) or satellite cells are main actors of muscle regeneration. Other resident stem cells in human skeletal muscles, called adipogenic progenitors (APs) have been recently discovered by us and other groups. Unlike MPs, they exclusively differentiate into adipocytes. No differentiated adipocytes are present in healthy young or adult muscles. However, fat infiltrations are observed in myopathies, obesity or aging, and are frequently associated with a chronic inflammation. Our aim is to elucidate APs role and regulation with the hypothesis that MPs and inflammatory mediators regulate APs in a non-pathological context and that this regulation is disrupted in diseased muscles.

Recent studies in mice have pointed out cross-talks between APs and MPs regulations. The first objective is to determine (i) factors secreted by MPs that regulate APs proliferation and differentiation, (ii) the role of APs in human muscle regeneration/degeneration. For investigations on interactions between APs and MPs, we developed a co-culture system wherein the differentiation medium allows both adipogenic and myogenic differentiations. In these cell interacting conditions, and only for progenitor cells derived from young donor muscles, we showed a stimulation of MPs myogenesis and an inhibition of APs adipogenesis. The stimulation of MPs differentiation seems to be dependant to cell-to-cell contacts with APs. To the contrary, conditioned medium experiments showed that inhibition of APs adipogenesis is mediated only by soluble factors. In addition, we observed an increase of APs proliferation in the presence of MPs conditioned medium and a decrease of MPs proliferation with APs conditioned medium. Finally, we screened a panel of soluble factors and three factors, HGF, IGF-1 and TNFα, were found to be important candidates as cytokines secreted by MPs and/or APs and responsible of APs/MPs interactions.

The inflammatory process is crucial for adult skeletal muscle repair, notably by acting on MPs. The second objective focuses on the role of factors secreted by macrophages in the regulation of APs proliferation and differentiation. To study interactions between macrophages with APs, the THP-1 human monocytic cell line was used. The THP-1 macrophagic differentiation was induced with phorbol myristate acetate and the conditioned medium of mature macrophages was collected to be added on APs cultures. APs proliferation and adipogenesis were significantly decreased, suggesting an essential role of inflammatory factors secreted by macrophages on APs regulation. Among the known factors secreted by macrophages, TNFα, TGFβ1 and Activin A inhibit APs differentiation.

Our results show (i) an inhibitory control of fat infiltrations by young healthy muscles and macrophages (ii) a specific function of APs in muscle regeneration. These new findings on the impact of APs on myogenic differentiation of MPs and the APs regulation by MPs and macrophages in healthy human muscle shed new lights on the role of the recently discovered APs, as well as a deeper understanding of muscle homeostasis.

Poster n°15

Effect of dual biotic stress on plant volatile synomones used by egg parasitoids

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

**ISA - Institut Sophia Agrobiotech, Sophia-Antipolis - UMR INRA 1355 - CNRS 7254 - UNS*

Studies on semiochemical communication have demonstrated that broad bean plant, *Vicia faba*, emits volatile synomones induced by feeding and oviposition activities of the southern green stink bug, *Nezara viridula*, which recruit the egg parasitoid *Trissolcus basalis*. However plants growing in agro-ecosystems can be attacked by several herbivore species that could affect both above and belowground plant tissues with possible consequences for parasitoid recruitment. For example, broad bean plants can also be attacked by the leaf weevil, *Sitona lineatus*, and simultaneous attacks by the southern green stink bug and the leaf weevil can occur in agro-ecosystems. The purpose of this work was to investigate the effects of dual biotic stresses, the host *N. viridula* and the non-host *S. lineatus*, on *V. faba* volatile synomones that recruit *T. basalis*. The response of wasp females to *V. faba* volatiles was investigated in a Y-tube olfactometer testing the following treatments: (1) plant damaged by *N. viridula* feeding and oviposition; (2) plant damaged by *S. lineatus* feeding; (3) plant mechanically damaged to resemble *S. lineatus* feeding; (4) plants damaged by *N. viridula* feeding and oviposition and by mechanical damages; (5) plants damaged by *N. viridula* feeding and oviposition and by *S. lineatus* feeding; (6); healthy plants. Volatile organic compounds emitted from tested plants were also chemically analyzed by Gas Chromatography –Mass Spectrometry (GC-MS). The results showed that dual biotic stresses affect *V. faba* volatile synomones decreasing their attractiveness towards *T. basalis*. Chemical analysis indicated qualitative 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. The ecological consequences of these results in terms of multi-trophic interactions are discussed.

Poster n°16

The transcription factor COUP-TFI regulates granule cell differentiation in the developing hippocampus

Joséphine Parisot, Gemma Flore and Michèle Studer*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

The nuclear receptor COUP-TFI acts as a strong transcriptional regulator in the developing neocortex and plays, among others, key roles in cell-type specification, axon elongation and cell migration. Yet, little is known about its involvement in the archicortex and particularly in the hippocampus, a major component of the mammalian brain playing crucial roles in learning and memory.

In this study, we found that COUP-TFI is expressed in proliferating and differentiating neural progenitors in the hippocampus, in granule cells during their migration and maturation and in the subgranular zone of the dentate gyrus, one of the two neurogenic regions of the adult brain. By inactivating COUP-TFI in the cortex from E10.5, the hippocampus is reduced and displaced, and the temporal progression of granule cell development is highly perturbed, resulting ultimately in decreased neurogenesis in adult COUP-TFI mutant mice. Careful analysis of the different steps of dentate gyrus granule cell differentiation in mutant hippocampi indicates premature astrogliogenesis at the expense of neurogenesis. This suggests that COUP-TFI acts in the neuron-glia cell fate decision during early stages of granule cell development. Together, these data indicate that COUP-TFI might be involved in regulating particular aspects of granule and stem cell neurogenesis, and propose it as a novel factor required in hippocampal development and cell maturation.

Poster n°17

The role of tumor cell-derived SPARC/AKT-mediated p53 regulation in resistance of melanoma cells to V600EBRAF inhibition

Pottier Anaïs, Fenouille Nina, Girard Christophe, Deckert Marcel, Tartare-Deckert Sophie.*

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

Oncogenic mutations in BRAF are the most represented in human melanoma, a lethal skin cancer with increasing incidence. To date specific V600EBRAF inhibitors (Vemurafenib or Dabrafenib) fail to cure the metastatic disease because many patients relapse due to development of mechanisms of resistance. The tumor microenvironment is often implicated in promoting drug resistance, and contributes to AKT pathway activation in tumor cells. We previously showed that a matricellular protein secreted by melanoma cells, SPARC, is responsible for elevated of AKT signaling and promotes an autocrine loop that confers survival advantage through suppression of p53-dependent apoptosis.

In this study we have investigated the interplay between SPARC, AKT signaling and p53 in primary and acquired resistance to BRAF targeted therapies. We found that overexpression of SPARC in V600EBRAF-mutated melanoma cells attenuates sensitivity to Vemurafenib and Dabrafenib and to chemotherapy-induced cytotoxicity. Conversely, knockdown of SPARC by siRNA cooperates with BRAF inhibitors to promote clonogenic cell death. Mechanistically, SPARC knockdown was found to decrease phosphorylation of AKT, which is associated with activation of p53. To better understand the role of SPARC in resistance to BRAF inhibitors, we analyzed its levels in V600EBRAF melanoma sublines with acquired resistance to Dabrafenib or Vemurafenib. We found that development of resistance is associated with increased SPARC and phosphorylated AKT, and a decrease in p53 protein level, which are reversed upon SPARC depletion by siRNA. Importantly, we found that extinction of SPARC expression resensitizes Vemurafenib resistant cells to a sub-lethal dose of Vemurafenib. Furthermore, inhibition of the BRAF target MEK with a sub-lethal dose of the U0126 compound induces apoptotic and clonogenic death of SPARC-depleted Dabrafenib resistant cells. Our findings indicate that the level of SPARC is a determinant of therapeutic sensitivity of melanomas. Furthermore this study emphasizes the interest of targeted inhibition of SPARC in combinatory strategies to optimize BRAF inhibitor sensibility or overcome BRAF inhibitor resistance.

Poster n°18

Impact of a new HIV protease inhibitor on adipose tissue

Christophe Ravaud, Stéphane Azoulay, Christian Dani and Annie Ladoux*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

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 the Lopinavir (LPV), and Nucleoside Reverse Transcriptase Inhibitors (NRTI).

However, its use has been hindered by many metabolic adverse side effects. Indeed patients treated with HAART therapy develop lipodystrophy, dyslipidemia and insulin resistance, indicating that the adipose tissue is preferentially targeted by this therapy.

LPV is one of the most efficient and prescribed drug in developed countries despite the adverse effects reported. Thus the discovery of new molecules efficient to treat HIV infection and presenting fewer side effects is very important to improve patient life. Darunavir (DRV) corresponds to a last generation of PI that seems to be better tolerated by patients as they present less metabolism disorders.

A better knowledge of the cells targeted by these molecules and of the adverse mechanisms they caused is of interest to adapt treatments to patients. However a comparison between the mechanisms involved in the adverse side effects of these two drugs has not been reported so far.

In the present study, we used human Multipotent Adipose Derived Stem Cells (hMADS) which are able to differentiate toward adipocytes and osteoblasts to investigate the effects of LPV and DRV on both adipose precursor cells and on differentiated cells.

Here we show that DRV did not impact adipose precursor cells proliferation and self-renewal, while LPV dramatically decreased their proliferation and their ability to grow at a single cell level.

In differentiated cells, as other IP, DRV accumulated in lipid droplets. LPV promoted ER stress and insulin resistance subsequent to IRS1 phosphorylation defects while unphysiological DRV concentration only gave similar results. Furthermore, LPV impacted mitochondrial function while DRV had no significant effect.

In addition LPV dramatically impaired adipose differentiation of hMADS cells while DRV did not impact this process.

All-together, our results indicate that DRV induced less alteration than LPV on both adipose precursor cells and adipocytes, preventing then adipose tissue integrity. Thus, DRV treatment should improve the quality of life of AIDS patients receiving HAART therapy.

Poster n°19

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, Bernard Mari, and Nicolas Pottier*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

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 (Cardenas, IS. et al. Plos Genetics 2013, 9:2). Interestingly, miR-199a-5p belongs to the miR-199a/214 gene cluster encoded by the DNM3os (Dynamin 3 Opposite Strand) transcript. TGFβ stimulation of lung fibroblasts enhanced the expression of DNM3os and miR-199a/214, 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 induce lung fibroblast differentiation into myofibroblasts. Next, we addressed the importance of miR-214 in the molecular events underlying TGFβ signaling. Transcriptomic analysis of miR-214 overexpressing lung fibroblasts led us to identify miR-214 targets that are clearly involved in the non-canonical TGFβ pathway. Using similar approaches, we also reported that miR-214 was a potent regulator of the HGF/COX-2/PGE2 axis, which is crucial for lung epithelial repair.

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. Finally, as aberrant lung expression of miR-199a and miR-214 has also been found in IPF patients, the inhibition of this cluster may represent a new effective therapeutic option for this devastating disease.

Poster n°20

Identification of novel mechanisms of miRNA targeting

Tekaya Nedra, Pinot Anthony, Repetto Emanuela, Hizir Zoheir, Trabucchi Michele*

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

MicroRNAs (miRNAs) are small non coding RNA about 22 nucleotides (nt) in length, which are involved in many cellular processes, including cell proliferation, differentiation, apoptotis, and inflammatory response. miRNAs are associated with Argonaute2 (Ago2) and control gene expression mainly at the post-transcriptional level by binding to cognate mRNAs via Watson-Crick base pairing using partial sequence complementarity to mediate degradation or translational block. Different softwares aiming to predict miRNA-target mRNAs, such as miRANDA, TargetScan and PicTar, use algorithms to detect miRNA binding sites based on sequence complementarity between miRNA seed (8 nt at the 5\' end) and target mRNA and on the evolutionary conservation of the target sequence. Although some experimentally validated miRNA-target mRNAs were successfully identified in the first place by using the support of these algorithms in combination with mRNA profile analysis, this approach misleads to about 60% of false negative and 60% of false positive targets. This is mainly due to two different causes: i) the use of microarray analysis of the transcriptome restricts the identification of degraded target-mRNAs; ii) such algorithms ignore the complexity of the system in vivo, including secondary structure and RNA binding-proteins occupancy of the target sequence, or cis-regulation of other post-transcriptional pathways fail to find correct target mRNAs due to different causes, such as the AU rich element mediated degradation pathway. Overall, this would imply the existence of additional cis elements beyond the complementarity between miRNA and target mRNA. Based on these observations, we hypothesize the occurrence of a binding motif signature in the region of the target mRNA surrounding the miRNA::mRNA binding site. We have definite this region as miRNA-microenvironment. Some examples from the literature would support a critical miRNA-microenvironment of about 50-100 nt surrounding the miRNA binding site, including HuR and PTB. In this study, I looked for RNA-binding proteins (RBPs) that bind the miRNA-microenvironment to co-regulate miRNA mode of action on cognate mRNAs, by either promoting the accessibility to the binding sites or to stabilizing the miRNA::mRNA duplex. To address this question, I performed a mass-spectrometry analysis on the Ago2-containing complex. Briefly, I immunoprecipitated Ago2 from cell extracts from the macrophage cell line, Raw264.7 cells previously partially digested with RNAseI to produce RNA fragments of about 150nt in length, corresponding to the miRNA-microenvironment. The immuno-pellet was then analyzed by mass-spectrometry. Interestingly, among the other proteins we identified two novel RBPs (here called protein X and Y because of patenting reasons) associated with Ago2. Both X and Y co-immunoprecipitate with miRNAs loaded into Ago2 and their association with Ago2 is RNA-dependent. Noteworthy, the association between Ago2 and these proteins is present in many other cell lines indicating a general mechanism. In my second phase of the PhD, I will perform a series of genome-wide experiments, including RNA profiling and HITS-CLIP, to investigate the function of X and Y in regulating miRNA-targeting mechanisms. I foresee, this project will result into newer versions of in silico miRNA target prediction tools with considerably improved sensibility and precision.

Poster n°21

Role of Wt1 in cardiac angiogenesis and function after myocardial infarction in adult mice

Baudouy D, Vukolic A, Lopez P, Wagner N, Wagner KD, Van Obberghen E

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Cardiovascular diseases are a major cause of mortality, mainly related to myocardial infarction (MI). Ischemic cardiomyopathy corresponds to pathological remodelling after MI, resulting in heart failure. Wt1 (for Wilms' tumor suppressor gene) controls epithelial to mesenchymal transition in epicardial cells, a key step in cardiac angiogenesis during embryonic development. After MI, epicardial cells are reactivated, Wt1 is re-expressed in coronary vessels and colocalised with angiogenic factors. We postulated that Wt1 was required for cardiac repair after MI.

Our objectives are to assess the importance of Wt1 in cardiac vascularisation and function after MI and to identify the genes and molecular pathways involved in Wt1 effects.

We use a model of conditional vascular expression of Wt1 in adult healthy mice (Tie2-Cre-ERT2 ;Wt1loxP+/+), in which Wt1 is inactivated by tamoxifen injection. We mimic MI by doing surgical coronary artery ligation in sedated mice. We compare 3 groups: mice with Wt1 knockout (KO), mice with normal Wt1 expression (no tamoxifen injection) and a control group to assess tamoxifen cardiac toxicity (Tie2-Cre-ERT2mice). In those animals, we perform functional analyses with echocardiography (cardiac function and dimensions) at 4 different time points: before MI, at MI acute phase, at reparation phase and at chronic phase after MI. Histological analyses on heart sections after MI will determine MI and cardiomyocyte size, vascular density, and cardiac fibrosis. Molecular analyses will determine RNA differential expression between our groups after MI.

Preliminary results show that Wt1 KO does not affect cardiac function before MI. At MI acute phase, it causes cardiac dilation and increases MI size. Then, we showed that Wt1 is necessary for cardiac repair after MI: when compared to mice capable of re-expressing Wt1,

Wt1 KO mice have more pronounced cardiac dilation and fibrosis, increased cardiomyocyte and MI size, and diminished contractility and vascular density. We identified potential Wt1 direct target genes, inserted their promoter sequences (with WT1 response elements) in plasmids and are currently performing transfection experiments (+/- WT1).

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 neoangiogenesis, is of utmost importance to find adequate treatments after MI. Wt1 could be an important actor in cardiac repair after myocardial infarction.

Poster n°22

MFN2 and mitochondrial DNA instability

*Berg Laetitia**

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

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 may be caused by mutations, acquired or inherited, in mtDNA or mainly in nuclear genes that code for mitochondrial components.

We focuse our interest on mitochondrial diseases with mtDNA instability, characterized by reduction of mtDNA copy number (depletion) or by accumulation of mtDNA deletions in post-mitotic tissues.

Nuclear genes responsible for mtDNA instability disorders mainly fall into three cathegories: 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 membranes and contributes to the maintenance of the mitochondrial network.

We have identified two missense mutations in the GTPase domain of Mfn2 in two families affecting the same amino acid but leading two different phenotypes associated with mtDNA instability. The first one corresponds to a late-onset optic atrophy with neurological signs and mtDNA deletions. The second one corresponds to a more severe phenotype in a child with mtDNA depletion. We want to understand mechanisms responsible for this mtDNA intability by comparing the impact of these two MFN2 mutations on mitochondrial function, respiratory chain activity and dynamics and then to define the links between mitochondrial fusion and instability of the mitochondrial genome.

Poster n°23

The coupling of disc size sensing mechanism and Dilp8 expression

*Boone Emilie**

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

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. Interestingly, dilp8 expression levels drops at the end of larval development suggesting a direct coupling between autonomous organ growth programs and dilp8 expression. Identifying signals that regulate dilp8 expression during normal development is therefore likely to provide a better 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 making it an ideal candidate for assessing organ size. 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 and sufficient 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. Importantly, 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.

Poster n°24

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

Miled Bourourou, Carine Nguemeni, Elsa Gouix, Carole Rovère, Camille Simonneau, Anne Renault, Noëmie Simon-Rousseau, Catherine Heurteaux and Nicolas Blondeau*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

Aims

Few neurological conditions are as complex and devastating as stroke. Because over 100 clinical studies have failed, there is an urgent need for new molecules that stimulate neuroprotection such as nutraceuticals and we have demonstrated an omega-3 polyunsaturated fatty acid, the α-linolenic acid (ALA), ability to trigger mechanisms leading to neuroprotection, artery vasodilatation and neuroplasticity. Furthermore, ALA supplementation prevented mortality and cerebral damage in a murine cerebral ischemia model. We thus hypothesized that ALA supplementation might facilitate post-stroke motor and cognitive function recovery.

Methods

ALA supplementation consisted in enriching in ALA a diet (containing three times more ALA than regular chows). After a 6-week diet, focal ischemia (30min) was induced in mice by occluding their middle cerebral artery. Motor deficits were assessed in the rotarod and pole tests during the first three weeks after stroke and cognitive deficits were assessed in the Morris water maze (MWM) test between days 14 and 25 post-stroke.

Results

ALA supplementation increases time spent on the rotarod from day 2 to day 4 post-stroke and reduces latency to reach the floor at day 3 in the pole test.

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 in the MWM test.

Conclusions

Our results suggest that ALA supplementation facilitates recovery of motor and cognitive neurological functions. Our preclinical research suggests that ALA supplementation reduces post-stroke mortality, neuronal damage and promote rehabilitation.

Poster n°25

Metalloprotease-mediated shedding of PLA2R1, the main membranous nephropathy auto-antigen

Guillaume Dolla, Gérard Lambeau*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

PLA2R1 (Receptor for secreted PLA2s) is the major antigen in the serious auto-immune kidney disease called membranous nephropathy (MN). 40% of MN patients undergo endstage renal

failure. Hallmarks of MN are circulating antibodies against PLA2R1 and immune complexes found in the glomerular basement membrane.

PLA2R1 is a 180kDa membrane protein particularly expressed at the podocyte surface in renal glomeruli. PLA2R1 signaling and physiological function in kidney are still unknown. PLA2R1 also exist as a soluble form produced by alternative splicing. The role of this soluble form could be to bind circulating PLA2s, and in MN this soluble form could be the one accumulating in immune complexes.

In order to understand better PLA2R1 physiological role and GEM etiology, we study the PLA2R1soluble form. We demonstrate here the existence of a new soluble form secreted through a shedding-type proteolytic maturation of the extra-cellular domain of PLA2R1 in culture medium from transfected HEK. Paralogs and orthologs are also shed. This shedding is also found to various degrees for paralogs and orthologs of PLA2R1. Thanks to a pharmacological approach, we show here that shedding of PLA2R1 is done by MMPs/ADAMs proteases. We also ascertained the cleavage site through N-terminal sequencing.

These data offer a better knowledge of PLA2R1 properties and may help understand GEM etiology.

Poster n°26

Conversion of delta-cells into beta-like cells

Druelle Noémie, Courtney Monica, Vieira Andhira, Gjernes Elisabet, Avolio Fabio, Ben-Othman Nouha, Napolitano Tiziana , Mansouri Ahmed, and Collombat Patrick*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

The mature pancreas is organized into two compartments with distinct functions: the exocrine pancreas is involved in the production of digestive enzymes and their transport into the duodenum via a branched ductal tree, whereas the endocrine compartment consists of highly vascularized functional units called islets of Langerhans. These contain five distinct cell subtypes, alpha-, beta-, delta-, epsilon- and PP-cells responsible for the secretion of glucagon, insulin, somatostatin, ghrelin and pancreatic polypeptide (PP), respectively.

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease affecting nearly 6% of the world population. This disease is characterized by the specific destruction of insulin-secreting beta-cells leading to chronic hyperglycemia, which, if left untreated, results in a wide range of vascular complications. Despite current therapies, patient with T1DM display a shorter life expectancy due to the inability to strictly regulate glycemic levels. Therefore, new approaches to functionally replace lost beta-cells in diabetics are of growing interest.

Recently, it has been shown that the misexpression of Pax4 in alpha-cells induces their continuous regeneration and conversion into beta-like cells. Here, we investigated whether delta-cells could also be converted into beta-cells in this context and found that, indeed, delta-cells can adopt an insulin+ cell identity.

Additionally, combining the Cre/Lox system with lineage tracing, we have also generated mice allowing the misexpression of Pax4 in delta-cells. Our results demonstrate a dramatic islet hypertrophy provoked by an insulin+ cell hyperplasia.

Poster n°27

Study of the Rac1 ubiquitylation pathway : OPTN is a new partner of the HACE1 E3-ligase involved in adhesion-mediated control of cell division.

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

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

Rac1 is a Rho GTPase that belongs to the Ras super-family. Rac1 controls a number of cellular processes ranging from the actin cytoskeleton organization to proliferation as well as inflammatory responses. Therefore deregulation or mutations of this protein are involved in a number of human diseases such as autoimmune diseases and cancer.
While studying the effect of a Rho GTPase-targeting toxin called CNF1, our team discovered in 2002 a novel way by which cells regulate Rac1, that consists of ubiquitylation of the active form of Rac1 (Rac1-GTP) followed by its targeting to the proteasome for degradation (Doye A. et al Cell 2002).
Recently, we identified HACE1 as the E3 ubiquitin ligase that catalyzes the ubiquitylation of Rac1-GTP. HACE1 has a main role as a tumor suppressor, and Rac1 is its only known functional target to this day (Torrino S., Visvikis O. et al 2011 Dev. Cell). Via its action on Rac1, HACE1 impacts cellular NADPH oxidase activity and R.O.S production. To better understand HACE1's role in cell homeostasis we performed two-hybrid screen experiments to identify potential interacting partners or novel targets of this protein. The protein Optineurin (OPTN) had the highest score of interaction with HACE1.
Mutations of OPTN are involved in a number of human pathologies, however its main cellular function is not well characterized. Our preliminary results showed that OPTN is not a target of HACE1's ubiquitin ligase activity, and does not alter HACE1's ubiquitylation activity toward Rac1. The aim of our research is to understand why and how does the HACE1/OPTN complex take part in cell function.
Our study has revealed that there's an OPTN/HACE1 complex that is implemented when cells have high levels of activated Rac1. We also demonstrate that OPTN controls specifically CCND1 translation without altering CCND1 transcription or degradation, whereas HACE1 regulates CCND1 transcription. More importantly, we show that OPTN, controls cell proliferation by impacting G1 to S phase progression.
We further demonstrate that OPTN is a new component of cell-extracellular matrix (ECM) adhesion structures. Our data show that OPTN is a regulator of cell adhesion to the ECM by influencing Myosin Light Chain II phosphorylation levels, therefore altering actin stress fiber formation and maturation of Focal Complexes into Focal Adhesion. We also determined that OPTN modulates CCND1 expression in response to integrin clustering and signaling. Since abnormal tissue stiffness greatly contributes to tumor tissue growth, we're aiming next at demonstrating whether OPTN controls cell proliferation in response to tissue and cellular tension increase, phenomena that are dependent on integrin and Rac1 signaling. Altogether, our work aims at studying whether the HACE1/OPTN complex represents a tumor suppressor complex where HACE1 controls the cellular REDOX balance while OPTN controls the tensional homeostasis, both cellular physico-chemical states being important for normal proliferation.

Poster n°28

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

R. Ghandour, D. Pisani, G. Beranger, J-C. Chambard, M Giroud, D. Langin, G. Ailhaud C. Duranton, M. Tauc et E-Z. Amri*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Introduction: 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.

We decided to study the effect of an excess of poly unsaturated fatty acids ω6 on brown adiocytes formation.

Methods: Our team developed a unique cell model for studying the latel stepss of human brown adipogenic differentiation, the hMADS cells (human-Multipotent Adipose-Derived-Stem). We studied the effect of arachidonic acid (ARA) or its metabolites, prostaglandins (PGs) of series 2 treatments of hMADS cells, on the differentiation process.

Results: Our data show different effects of the prostaglandins studied. Thus, PGF2α and PGE2 inhibit the conversion of white adipocytes into brown adipocytes through a pathway involving intracellular calcium, MAPK and PPARγ. Meanwhile, PGE 2 is 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) induces this conversion through a pathway involving PPARs and IP receptor.

Conclusion: These results show that the effect of ARA on the conversion of white adipocytes 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 the adipocyte's membrane.

Our results suggest that in addition to promoting the formation of white adipocytes, excess of polyunsaturated fatty acids in diets may increase their deleterious effect, altering the process of \"browning\" in the white adipose tissue.

Poster n°29

Study of the role of the Parkinson's disease gene, PARK6, in brain cancer

Thomas Goiran, Eric Duplan, Julien Viotti, Jérôme Honnorat, Frédéric Checler and Cristine Alves da Costa*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

Gliomas are the most frequent and aggressive type of brain cancer and represent 70% of primary brain tumors. Unfortunately, the only available therapies are just palliative and do not really heal the disease. Gliomas are characterized by an excessive brain cells proliferation and cell death control impairment. The etiology of gliomas is tightly linked to a dysfunction of the master piece tumor suppressor, p53. Parkinson disease (PD) is the second most frequent age-related neurodegenerative pathology after Alzheimer's disease and is characterized by a massive dopaminergic neuron loss by exacerbated apoptosis. Interestingly, the increased cell death observed in PD is tightly linked to p53 accumulation. So, p53 is a key molecular player in both Parkinson disease and brain tumors. Interestingly, several epidemiological data evidenced a negative correlation between PD and cancer. This negative correlation raises the question of whether despite phenotypically distinct, these two diseases could share common protein effectors other than p53. 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 we have decided to investigate the interplay between p53 and Pink-1 in gliomagenesis. PINK1 is a serine threonine kinase associated to autosomal recessive PD which has been implicated in the control of mitochondrial integrity and function. We demonstrate that overexpressed and endogenous p53 can modulate pink-1 levels in several ex-vivo models and that this interplay is abolished by p53 hot spot mutations. Moreover, by means of adenovirus mediated delivery of wild-type and mutated p53 in mice brain we demonstrate that the regulation of pink-1 by p53 is also present in an integrated in vivo model. In conclusion, this study demonstrates for the first time a molecular link between p53 and pink-1 ex-vivo and in vivo and reinforces the possibility of a role of pink-1 in brain tumors development.

Poster n°30

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 Hübner, André W. Brändli, and Andreas Schedl*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Kidney organogenesis requires the tight control of proliferation, differentiation, and apoptosis of renal progenitor cells. How the balance between these cellular decisions is achieved remains elusive. The Wilms' tumor suppressor Wt1 is required for progenitor survival, but the molecular cause for renal agenesis in mutants is unknownpoorly understood. Here we demonstrate that lack of Wt1 abolishesd FGF and inducesd BMP/pSMAD signaling within the metanephric mesenchyme. Addition of recombinant FGFs or inhibition of pSMAD signaling rescuesd progenitor cell apoptosis induced by the loss of Wt1. We further show that recombinant BMP4, but not BMP7, induces an apoptotic response within the early kidney that can be suppressed by simultaneous addition of FGFs. This data reveals a hitherto unknown sensitivity of early renal progenitors to pSMAD signaling, establishes FGF and pSMAD signaling as antagonistic forces in early kidney development and places WT1 as a key regulator of pro-survival FGF signaling pathway genes.

Poster n°31

Thalamo-cortical hyperexcitability & High Frequency Oscillations (HFOs) induced by hypoexcitability of GABAergic neurons in a GEFS+ NaV1.1 Knock-In mouse model

Jennifer Lavigne, Camille Liautard, Ulrike Hedrich, Daniel Kirschenbaum, Andrew Escayg, Holger Lerche and Massimo Mantegazza*

**IPMC - Institut de Pharmacologie Moléculaire et Cellulaire, Sophia-Antipolis - CNRS UMR 7275 - UNS*

Epilepsies are chronic neurologic disorders affecting up 1% of people worldwide with major implications not only for health but also for independent living. They are characterized by recurrent seizures, often because of an imbalance between excitation and inhibition of neuronal networks, resulting in excessive and abnormally synchronized neuronal discharges.

The SCN1A gene, which codes for Nav1.1 sodium channel, is the most common gene affected in genetic epilepsy disorders.

Our study reveals that brain slices of the Knock-In Nav1.1-R1648H mouse model of Genetic Epilepsy with Febrile Seizures Plus (GEFS+) show a generalized impairment of excitability of inhibitory neurons and of GABAergic transmission in the thalamo-cortical loop, inducing hyperexcitability and epileptiform discharges, which may generate seizures *in vivo*.

Poster n°32

Titre manquant

*Elodie Long**

**CHU - Centre Hospitalier Universitaire, Nice*

Abstract non fourni

Poster n°33

Post-mitotic control of sensory areal specification during neocortical development

Magrinelli E., Alfano C., Harb K., Hevner R. F. and Studer M.*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

The process of neocortical arealization involves a series of events that allow the correct formation of functional areas during embryonic and early post-natal development. Areal identity is specified by graded expression of patterning genes both in neuronal progenitors and in neurons. However, whether post-mitotically expressed genes just maintain areal properties previously specified in progenitors or independently define them in committed neurons, is still unclear. COUP-TFI, a transcriptional regulator expressed in a caudolateral-high to rostromedial-low gradient in both progenitors and post-mitotic cells, plays a key role during neocortical areal patterning by promoting sensory and repressing motor areal identity during early corticogenesis.

Here we investigate whether COUP-TFI is sufficient to control areal patterning solely in post-mitotic cells. We used genetic loss- and gain-of-function approaches in mice and independently challenged COUP-TFI function in cortical progenitors or in neurons. Absence of COUP-TFI solely in post-mitotic neurons leads to severe areal phenotypes similar to the ones described after its inactivation in all cortical cells. Reintroducing COUP-TFI expression specifically in post-mitotic cells of constitutive COUP-TFI null mice remarkably rescues their areal and laminar defects. In addition, ectopic (post-mitotic) high expression of COUP-TFI in frontal/motor regions is sufficient to reprogram neurons from a motor to a sensory fate.

This study demonstrates that COUP-TFI is necessary and sufficient to drive cortical sensory identity in post-mitotic neurons independently of its early expression in progenitors. Overall, our data provide some direct evidence of a crucial role for post-mitotic patterning genes in areal specification and reveal an unexpected plasticity in this process, which may account for the complex and evolutionarily novel structures characteristic of the mammalian neocortex.

Poster n°34

euL1db: The European database of L1-HS retrotransposon insertions in humans

Mir AA, Philippe C, Cristofari G*

**IRCAN - Institute for Research on Cancer and Aging, Nice - CNRS UMR 7284 - INSERM U 1081 - UNS*

Retrotransposons are highly repetitive and dispersed sequences. These mobile genetic elements have the ability to proliferate via an RNA-mediated copy-and-paste mechanism, called retrotransposition. In humans, the long interspersed element-1 (L1 or LINE-1) is the only autonomous transposable element able to generate new copies in the modern human genome. The role of retrotransposition as a source of genetic diversity and diseases in humans has been shown by many studies. Advances in deep-sequencing technologies have shed a new light on the extent of L1-mediated genome variations. They have also lead to the discovery that L1 is not only able to mobilize in the germline - resulting in inheritable genetic variations - but can also jump in somatic tissues, such as embryonic stem cells, neuronal progenitor cells, or in many cancers. Understanding the link between L1 retrotransposon insertion polymorphisms (RIP) and phenotype or disease has become an intense field of research. Therefore there is an increasing need to catalogue and annotate the exploding number of germline and somatic L1 insertions in a common and curated repository. We designed and implemented euL1db to bridge this missing link. euL1db provides a curated and comprehensive summary of L1 retrotransposon insertion polymorphisms identified in healthy or pathological human samples and published in peer-reviewed journals. It currently contains >150'000 L1 insertion polymorphisms in >800 samples and >500 individuals for a total of >20'000 non-redundant distinct insertions. It can be searched and browsed through multiple criteria (genomic location, gene, RIP, study, sample, individual, family). When available, sample and individual entries contains clinical and geographical information. An important feature of euL1db is that insertions can be retrieved at a sample-by-sample level to facilitate correlations between the presence/absence of an L1 insertion with a specific phenotype or disease. A number of tools are also included to explore, visualize and extract euL1db data. Therefore, euL1db will be a useful resource not only for the large transposable element community, but also for the broad field of human genomics.

Poster n°35

Where, when and how many: introduction strategies of organisms in a spatially structured environment

Thibaut Morel-Journel, Ludovic Mailleret, Elodie Vercken*

**ISA - Institut Sophia Agrobiotech, Sophia-Antipolis - UMR INRA 1355 - CNRS 7254 - UNS*

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 a model of population dynamics in a spatial context, describing the migration of individuals and their reproduction according to parameters related to landscape structure. Predictions about the impact of different landscape features on establishment could then be tested by experiment. 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.

Poster n°36

Human induced pluripotent stem cells from Andersen's syndrome patients: Implication of a potassium channel in bone morphogenesis

Pini J., Rouleau M., Sacconi S., Desnuelle C., and Bendahhou S.*

**LP2M - Laboratoire de PhysioMédecine Moléculaire, Nice - CNRS UMR 7370 - UNS*

Andersen's syndrome is a rare disorder characterized by a triad of symptoms: periodic paralysis, cardiac arrhythmia and dysmorphic features. Patients with Andersen syndrome have bone developmental defect as craniofacial features, dental and skeletal anomalies. Most of the patients have a mutation in the KCNJ2 gene that encodes for the inward rectifier potassium channel Kir2.1. This mutation leads to a loss of function with a dominant-negative effect.

Kir 2.1 channels play a major role in maintaining the rest membrane potential in excitable cells such as cardiac and skeletal muscle cells. The electrical characteristics of mutations in Kir2.1 are well known but the implication of this channel in the bone development is actually unknown. KCNJ2 knockout mice are born with a cleft palate and die few hours after birth preventing further investigations. To investigate the molecular determinants that require Kir2.1 channels in bone morphogenesis, we use human myoblasts to generate induced pluripotent stem (iPS) cells.

The iPS cells are adult cells, which are reprogrammed to an embryonic stem cells state. These cells could proliferate without differentiation and give all cell types from the three germ layers.

We have generated iPS cells from healthy as well as Andersen's syndrome patient muscular biopsies (taken from Vastus lateralis). We infected adult patient myoblasts with lentivirus, which contain the 4 genes required reprogramming (Oct4, Sox2, Klf4 and cMyc). The cells obtained after infection have the characteristics of classical iPS cells and embryonic stem cells. They express pluripotent, genetics, as well as surface markers. These cells could also differentiate into the three germ layers by the embryoid bodies formation and express specific markers of ectoderm, endoderm and mesoderm.

To go further in the bone development, we differentiated patient and control iPS cells into the mesodermal pathway, and mesenchymal stromal cells obtained from both iPS cells exhibit the same characteristics.

Altogether, our results show that Kir2.1 channels are not important for the reprogramming process and for the differentiation into mesenchymal stromal cells.

The iPS cells from Andersen patients may provide a good tool to study the implication of the Kir2.1 potassium channels in bone development.

Poster n°37

Study of antitumor potential of calcium phosphate cements loaded with bisphosphonate or Sunitinib on bone metastsis development

Al-Sahlanee Rasha, Goguet-Surmenian Emilie, Millet Marie-Ange, Scimeca Jean-Claude, Schmid-Antomarchi Heidy, , Schmid-Alliana Annie*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Bone metastases are a frequent complication of cancer and pose a significant public health problem.Bone represents one of the most common site of cancer metastasis from breast and kidney cancers, in particular, tend to metastasize to bone sites with high frequency. The bone invasion by malignant cells rapidly compromises the balance between synthesis/ mineralization of bone matrix (performed by osteoblasts) and bone resorption (performed by osteoclasts), and leads then to the development of bone metastases classified either as osteoblastic or osteolytic. In kidney and breast cancers, bone metastases are predominantly osteolytic. These bone lesions ultimately cause a dramatic bone loss resulting in skeletal-related events (SREs) such as severe bone pain, bone instability, fractures, spinal cord compression, hypercalcemia and bone marrow aplasia.

Currently, several clinical strategies are employed in order to manage bone metastasis, including surgery, radiation, chemotherapy and treatments with targeted agents. However, the prognosis for a patient with bone metastases remains poor, since neither of these strategies improves the patient's survival, prompting the research of new therapeutic strategies.

Bisphosphonates (BPs) and VEGF inhibitors such as Sunitinib, are used as targeted agents in the treatment of breast and kidney cancer's bone metastases. These agents, used by oral or systemic approaches, cause many severe side effects. Unfortunately, occurrences of these side effects often lead the clinician or even the patient himself, to reduce or interrupt the treatment, therefore compromising its efficiency.

When bone metastases occur in the long bones, as these bones represent locations mechanically exposed to the risk of fractures, they often involve surgical resections followed by filling of the bone defect thus created. In this context, a bone-targeted delivery of these molecules (BPs and/or Sunitinib) appears as an interesting strategy in order to reduce the administered dose and thereby reducing their side effects while retaining their antiresorptive and/or antitumor potential. In this perspective, we chose resorbable calcium phosphate cements which are frequently used as a biomaterial implant in filler for bone defects, as a vector material of BPs and Sunitinib, in order to locally deliver these therapeutic agents. The grafted biomaterials will be synthesized and supplied by Graftis Company (Nantes, France) with whom our team has worked for many years. The characterization of cellular and molecular mechanisms will be performed by in vitro and in vivo approaches. The techniques used to develop this project involve cancer animal models, molecular and cellular biology, immunohistochemistry and in vivo imaging.

Ultimately, our goal is to propose the use of grafted biomaterials in clinic in order to consolidate and locally address the bone defects created by tumor surgical resection and to prevent local recurrence.

Poster n°38

Implication of E2F1 transcription factor in melanoma

Florian Rouaud, Patricia Abbe, Michaël Cerezo, Abdelali Lehraiki, Stephane Rocchi*

**C3M - Centre Méditerranéen de Médecine Moléculaire, Nice - UMR INSERM U 1065/UNS*

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/MDM2 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.

Poster n°39

Rspondins are Required for Adrenal Gland Homeostasis

Sonia Zaddam, Roberto Bandiera, Valerie Vidal, Fariba Ranc, Bruno Ragazzon, Jérôme Bertherat, Hitoshi Okamoto, Antoine Martinez, and Andreas Schedl*

**IBV - Institute of Biology Valrose, Nice - CNRS UMR 7277 - INSERM U 1091 - UNS*

Wnt/β-catenin signaling is a key pathway in organ development and tissue homeostasis and is implicated in proliferation and differentiation of stem cells. Constitutive activation of β-catenin is the most frequent event that induces adrenocortical tumors. We are interested in Rspondins, a family of proteins that have been reported to act as activators of canonical Wnt/ß-catenin signaling. Our analyses show that expression of Rspo1 and Rspo3, two members of this family, are maintained within the adrenal capsule throughout life, a site that has been proposed to contain a stem/progenitor cell compartment. To address the role of Rspondins in development and homeostasis we performed loss-of-function approaches using genetically modified mice. Analysis of Rspo1 knockout mice did not reveal an overt adrenal phenotype. In contrast, deletion of Rspo3 at embryonic day E11.5, lead to significantly smaller adrenals exhibiting a disrupted cortex structure at E16.5. Moreover, removing Rspo3 in adult mice caused a reduction of cortex size within 6 weeks after deletion suggesting a requirement for this gene in adrenal homeostasis. To complement these loss-of-function studies, we ectopically expressed Rspo1 within the developing adrenal cortex. Strikingly transgenic mice displayed dramatic overgrowth of the adrenal cortex as early as E15.5. Interestingly, the size of the adrenal cortex increased in a time- and dosage-dependent manner without an overall disturbance of the cortical architecture. At later stages of life (>6 months), however, a proportion of mice developed adreno-cortical tumors. Taken together, these data demonstrate an essential role for Rspo3 in adrenal gland development and homeostasis and reveal a growth promoting and oncogenic capacity of Rspondins. Our results might also suggest that overexpression of these genes may be at the basis of adrenocortical tumors in human patients.

PROGRAMME

jeudi 4 septembre 2014

jour entier

08:00

08:15
Accueil des participants

09:00

Ouverture des JEDNs par Thomas Lamonerie, Président de l'ED85

Présentations orales des doctorants en 3ème année

10:00

09:45
Conférence du Dr Anne TSICOPOULOS : CCL17 production by dendritic cells is required for NOD1-mediated exacerbation of asthma

Pause

11:00

Présentations orales des doctorants en 3ème année

11:15
Conférence du Pr Marc-André SELOSSE : Mycoheterotrophy and mixotrophy: plants eating mycorrhizal fungi.

12:00

"Mon poster en 60 secondes"

12:15
Pause repas

13:00

13:15
Session Posters (n°1 à 20) - Doctorants en 2ème année + Atelier

14:00

15:00

Présentations orales des doctorants en 3ème année

15:15
Conférence du Dr Serge LUQUET : Dietary triglycerides act on mesolimbic structures to regulate the rewarding and motivational aspects of feeding

16:00

Pause

16:30
Présentations orales des doctorants en 3ème année

17:00

17:30
Table Ronde : La création d'entreprise après la thèse

18:00

18:30
Apéritif et Repas

19:00

20:00

21:00

22:00

vendredi 5 septembre 2014

08:00
Accueil des participants

08:45
Présentations orales des doctorants en 3ème année

09:45
Conférence du Dr David GREMILLET : Spatial ecology of seabirds; from microelectronics to the conservation of the species

Pause

11:00
Présentations orales des doctorants en 3ème année

"Mon poster en 60 secondes"

12:00
Pause repas

12:45
Session Posters (n°21 à 39) - Doctorants en 2ème année + Atelier

14:15
Présentations orales des doctorants en 3ème année

15:00
Conférence du Pr Dominique SCHNEIDER : Long-term experiment with Escherichia coli: is evolution repeatable?

Pause

16:15
Présentations orales des doctorants en 3ème année

Remise des prix