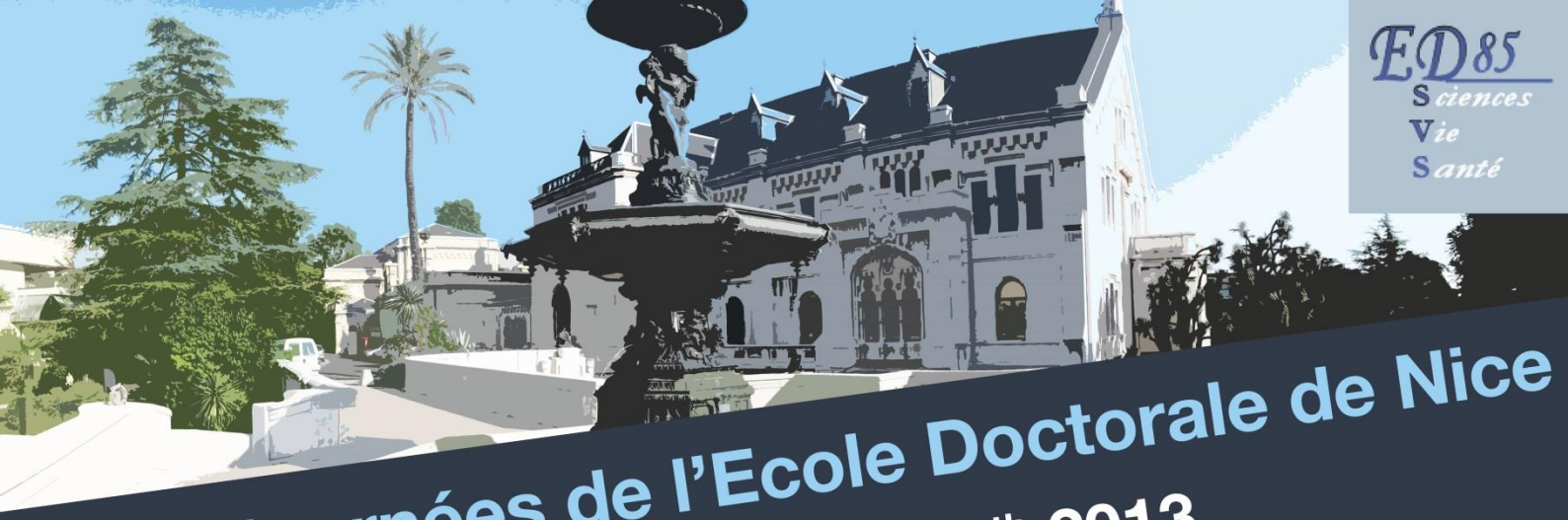

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Invités

David Biron

Laboratoire "Microorganismes : Génome et Environnement", Université Blaise Pascal, Aubière

« La manipulation parasitaire : un mythe ou une réalité ?»

Ingrid Bethus

Section cerveau, cognition et comportement, IPMC, Nice

« Titre à confirmer »

Francois JUGE

RNA metabolism, Institut de génétique moléculaire de Montpellier

« Le facteur d'épissage B42/SRp55 module la transcription et la croissance cellulaire chez la drosophile »

Marc DAERON

Département d'Immunologie, Institut Pasteur, Paris

« Des anticorps, pour le meilleur et pour le pire »

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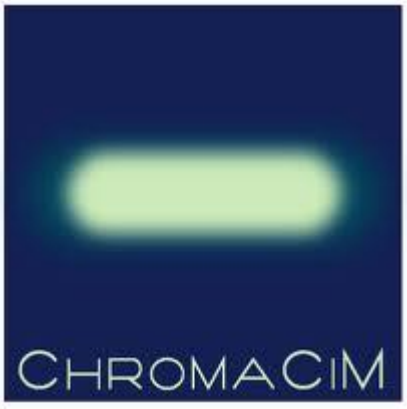

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Nicolas CALLEMEYN

JEDNs 2013 : Programme

Jeudi 5 Septembre

8h00-8h45 **Accueil des participants**

8h45-9h **Ouverture des JEDNs par Thomas Lamonerie**

9h-9h15 **Edouard EVANGELISTI**

Penetration-Specific Effectors from the Oomycete Phytophthora parasitica Modulate Plant Defense Responses and Interfere with Plant Development and Physiology.

9h15-9h30 **Geordie ZIMNIAK**

A novel blood cell-niche association controls Collagen IV and basement membrane assembly, essential for germline stem cell homeostasis in Drosophila melanogaster.

9h30-9h45 **Gérald BERMOND**

Absence of effect of admixture between two European invading outbreaks of a major corn pest in natura.

9h45-10h00 **Julie VEYSSIERE**

Spadin is devoid of side effects on TREK-1 controlled functions.

10h00-10h30 Pause café

10h30-10h45 **Anja PFEIFER**

Duct-lining cells can reprogram into beta-like cells able to counter repeated cycles of toxin-induced diabetes.

10h45-11h **Nicholas DUBOIS**

The deubiquitinating enzyme USP14 controls MCL-1 expression and leukemic cell survival.

11h-11h15 **Hervé QUINTARD**

The Chinese medicine neuroaid (MLC601, MLC901) induces potent neuroprotective and neuroproliferative effects after global cerebral ischemia and traumatic brain injury.

11h15-12h ***David Biron***

Laboratoire "Microorganismes : Génome et Environnement", Université Blaise Pascal, Aubière.

« La manipulation parasitaire : un mythe ou une réalité ?»

12h-12h45 Pause déjeuner

12h45-14h **Présentation des posters: session 1.**

14h-14h45 ***Ingrid Bethus***

Section cerveau, cognition et comportement, IPMC, Nice.

« Titre à confirmer »

14h45-15h **Julien VIOTTI**

Study of the role of the AR-JP associated protein parkin in the development of human brain tumors.

15h-15h15 **Sébastien VIOLLET**

Purification and characterization of ribonucleoprotein particles associated with the L1 ORF1p protein.

15h15-15h30 **Hugo MATHE-HUBERT**

Variability and evolution of the venom of two biological control agents from the Psyttalia genus.

15h30-16h Pause café

16h-16h15 **Sabiha ABEKHOUKH**

Study of the interaction between FMRP and GRK mRNA.

16h15-16h30 **Christian ROUX**

Osteoarthritis: from epidemiology to fundamental: Chondrogenic potential of stem cells derived from adipose tissue and ocytocine action on chondrocyte.

16h30-16h45 Andhira VIEIRA

Inducible misexpression of Ngn3 in pancreatic duct-lining cells.

16h45-17h Damien BARNEAUD-ROCCA

Structural model of the anion exchanger 1: idenfication of the transport site.

17h-17h15 Jérome DOYEN

Occurrence of metastasis and in vitro aggressiveness of triple negative breast cancer is related to the glycolytic phenotype, microenvironment metabolism and MCT4 lactic acid transporter.

17h15-18h15 Après Thèse.

CJCAM.

Laurence Dupont : recrutement maitre de conférences universitaire.

Thomas Guillemaud : recrutement INRA.

Jean-François Tanti : recrutement INSERM.

18h30 Apéritif dinatoire / Soirée de Gala.

Vendredi 6 Septembre

8h30 9h **Accueil des participants**

9h-9h15 **Tanesha NAIKEN**

Metabolism and cancer: exploration of a potential metabolic cooperation between tumor associated macrophages and carcinoma cells.

9h15-9h30 **Mickael CEREZO**

Metformin Blocks Melanoma Invasion and Metastasis Development in AMPK/p53-Dependent Manner.

9h30-9h45 **Franck CEPPO**

Implication of the inflammatory kinase Tpl2 in the deleterious effects of cytokines and macrophages on adipocytes functions.

9h45-10h **David CROTTES**

Sigma-1 receptor (Sig1R) regulates tumor / extracellular matrix interactions through modeling electrical plasticity – consequences on cancer progression.

10h-10h30 Pause café

10h30-10h45 **Kawssar HARB**

A novel molecular mechanism specifying different subpopulations of layer V neurons projecting to the pons or spinal chord in the developing mouse cortex.

10h45-11h **Jean ALBRENGUES**

Production of LIF cytokine by cancer cells and fibroblasts contributes to the establishment of a pro-invasive tumor microenvironment.

11h-11h15 **Audrey TOUZOT**

The role of the nuclear receptors COUP-TFs in the migration and the specification of cortical interneurons during mouse development.

11h15-12h ***Francois JUGE***

RNA metabolism, Institut de génétique moléculaire de Montpellier.

« Le facteur d'épissage B42/SRp55 module la transcription et la croissance cellulaire chez la drosophile »

12h-12h45 Pause déjeuner.

12h45-14h **Présentation des posters: session 2.**

14h-14h45 ***Marc DAERON***

Département d'Immunologie, Institut Pasteur, Paris.

« Des anticorps, pour le meilleur et pour le pire »

14h15-15h **Eva PAUPER**

Functional analysis of the RSPO1/beta-catenin signaling pathway in normal ovarian development and in ovarian pathologies.

15h-15h15 **Imene HENAOUI**

MiR-199a-5p, novel effector of the TGFbeta pathway.

15h15-15h30 **Bruno FANT**

The role of Otx2 in the correct positioning of the midbrain-hindbrain barrier.

15h30-16h Pause café

16h-16h15 **Lucas MOURGUES**

The polycomb protein BMI1 promotes cell proliferation through repression of cyclin protein expression in Chronic Myeloid Leukaemia Cells.

16h15-16h30 **Joffrey PELLETIER**

Cancer metabolism: exploiting disruption of AMPK and glycolysis as a potential therapeutic approach.

16h30-16h45 **Arnaud BARBARY**

Towards the deciphering of the genetic factors involved in durability of plant major resistance genes to root knot nematodes in pepper.

16h45-17h **Stéphanie PATOURAUX**

Use of hepatocyte death markers for the prediction of non alcoholic steatohepatitis in morbidly obese patients.

17h-17h15 **Pierre-Jean CORNEJO**

Implication of microRNA-34a in adipocyte insulin resistance during obesity.

17h30-18h **Remise des prix**

Présentations Orales

Oral presentation

Penetration-Specific Effectors from the Oomycete Phytophthora parasitica Modulate Plant Defense Responses and Interfere with Plant Development and Physiology.

EVANGELISTI Edouard[1], COMBIER Maud[1], MINET-KEBDANI Naïma[1], KUHN Marie-line[1], ATTARD Agnès[1], PANABIÈRES Franck[1], GOURGUES Mathieu[1]

1: UMR Institut Sophia Agrobiotech, INRA 1355, CNRS 7254

Keywords: oomycetes, phytophthora parasitica, effector, penetration, development, hormone, root

Oomycetes are fungus-like microorganisms that display strong morphological similarities with fungi. However, they belong to the kingdom of Stramenopila, together with brown algae and diatoms. Oomycetes include detrimental pathogens of animals and plants. Plant pathogenic oomycetes infect a wide range of crop species and ornamental plants and cause serious damages to natural ecosystems. Among them, the most prominent plant pathogens belong to the genus Phytophthora. The molecular events which occur during the penetration of the first host cells are of pivotal importance since they guide the outcome of the interaction toward plant resistance or disease installation. Molecular exchanges between the two partners include the secretion of small molecules termed effectors that allow plant pathogens to interfere with host cell structure and/or function. During my PhD, I performed the functional analysis of three effectors from wide-host-range species Phytophthora parasitica that transiently accumulate during the penetration process. These effectors, referred to as penetration-specific effectors 1, 2 and 3 (PSE1, PSE2 and PSE3), significantly increase Arabidopsis thaliana susceptibility to P. parasitica infection and suppress plant defense responses. Effector expression in A. thaliana showed that PSE1 and PSE2 also interfere with plant development. Constitutive accumulation of PSE1 led to altered root and root hair development which is associated with modulation of plant hormone physiology. PSE2 accumulation in A. thaliana led to strong perturbation of root anatomy, also suggesting alteration of root development. These results suggest that penetration-specific effectors contribute to the success of infection by targeting key plant functions such as hormone physiology. They may act as a relay and prepare the secretion of a new wave of effectors when the interaction is established.

Oral presentation

A novel blood cell-niche association controls Collagen IV and basement membrane assembly, essential for germline stem cell homeostasis in Drosophila melanogaster.

ZIMNIAK Geordie[1], VAN DE BOR Véronique[1], JUAN Thomas[1], CEREZO Delphine[1], NOSELLI Stéphane[1]

1: Institut de Biologie Valrose, University of Nice Sophia Antipolis, UFR Sciences,UMR7277iBV

Keywords: collagen iv, stem cell

The Basement Membrane (BM) provides essential structural support for epithelia and organ morphogenesis. Recent studies have shown that Collagen IV, a major BM component, is able to actively modulate cell signaling. Our lab uses the ovarian follicle in Drosophila melanogaster to study the function of Collagen IV during development. Ovarian follicles are made of germ line cells surrounded by a monolayer epithelium. Oogenesis is a highly active and dynamic process in which ovarian follicles are produced steadily from the germ line stem cell niche, further differentiating into different cell types. Using new specific markers developed in the lab, cell biology techniques and targeted genetic approaches, we could show that ovarian collagen IV originates from three different cell types: the adult fat body cells, the follicular epithelial cells, and from previously uncharacterized cells scattered in the ovary. These cells correspond to a new population of Drosophila blood cells or hemocytes. These “ovarian hemocytes” are preferentially located around the germarium, a structure housing the adult germline stem cell niche. Using controlled expression of fluorescently tagged forms of collagen IV, we show that hemocytes specifically deposit collagen IV around the germarium. Furthermore, our data show that the interaction between hemocytes and the germarium starts during larval stages and plays a crucial role in the maintenance of the germline stem cell niche. Preventing collagen IV production by the hemocytes, using RNAi or inducing hemocyte apoptosis, severely impairs the number of stem cells, mimicking a loss of decapentaplegic/TGF-beta activity, whose activity is essential to control the stem cells population. These results reveal a yet unknown interaction between circulating blood cells and the germline stem cell niche to control homeostasis and the rate of egg chamber production. We are further investigating the signaling pathways involved in this association.

Oral presentation

Absence of effect of admixture between two European invading outbreaks of a major corn pest in natura.

BERMOND Gérald[1], CAVIGLIASSO Fanny[1], MALLEZ Sophie[1], GUILLEMAUD Thomas[1]

1: UMR Institut Sophia Agrobiotech, INRA 1355, CNRS 7254

Keywords: biological invasions, rate of hybridization, microsatellite loci, diabrotica virgifera virgifera, populations genetics, heterosis

The reduction of genetic diversity that often occurs during introduction of invasive populations can decrease the adaptive potential of introduced populations. Multiple introductions followed by hybridization events may increase the genetic diversity and therefore facilitate invasion. In this study, we challenged the hypothesis that hybridization may have had a positive impact on the invasion of the western corn rootworm (WCR), Diabrotica virgifera virgifera. This beetle was introduced several times in Europe since the 1980's from the USA. The multiple introductions of this major pest of cultivated corn led to the formation of two major outbreaks that eventually merged into a secondary contact zone where insects from both outbreaks interbreed. We collected about 600 insects from this contact zone and genotyped them using 13 microsatellites. We then performed a Bayesian analysis of the population genetics structure in order to determine the hybrid status of each individual and its rate of hybridization (ROH). Finally, we statistically tested the hypothesis i) of a link between individual hybrid status or ROH and their mating success and ii) a link between individual hybrid status or ROH and their survival in stressful conditions. Our results show that the hybrid status or ROH does not impact the mating success or the survival of individuals. We conclude that this particular zone of admixture is probably neutral and will thus vanish through time under the effect of WCR dispersal.

Oral presentation

Spadin is devoid of side effects on TREK-1 controlled functions.

VEYSSIERE Julie[1]

1: IPMC Sophia Antipolis

Keywords: depression, trek-1, spadin, behavior, side effects

Introduction The two pore-domain potassium channel TREK-1 has been identified as a new target in depression and it has been hypothesized that TREK-1 antagonists might be effective antidepressants. We identified spadin, a peptide derived from the maturation of the neurotensin receptor 3 (NTSR3 /Sortilin), that specifically blocks TREK-1 channel. TREK-1 is an important channel involved in a number of other physiopathologies like pain, ischemia and epilepsy. Spadin displays the two main properties of antidepressant drugs, it increase both neurogenesis and serotoninergic transmission. But spadin offers the advantage to produce these effects more rapidly than classical antidepressants, four days instead of 21 days. We demonstrated that spadin did not affect these TREK-1 channel's functions. Spadin was also able to reverse depressive state in two depression models of mice (Corticosterone model and Rouen mice). Methods Spadin's side effects were studied through different tests: in the field of pain, we used the tail flick test, in the ischemic field, the model of focal ischemia and in the epilepsy field, kaïnate induced seizures (or PTZ induced seizures). We also investigated the effect of spadin at the cardiac level mainly on two important repolarizing currents (Ikr and Iks). The efficacy of spadin was studied by the use of two depression models developed in mice, an induced model with corticosterone treatment and the genetic model of Rouen mice. Results and discussion The specificity of spadin was demonstrated by electrophysiological measurements, we showed that spadin was specific for TREK-1 channels and did not modify the activity of four other K2P channels (TREK-2, TASK, TRAAK, TRESK). Interestingly spadin induced no side effects on functions controlled by TREK-1 channels. Spadin did not amplify the epileptic seizures and did not increase the thermal pain sensitivity. Even after a long term treatment spadin did not increase the size of brain infarct induced by the model of focal ischemia. The efficiency of spadin was also validated on two models of depression developed in mice. Spadin was able to increase the immobility time of depressed mice from both models of depression. Our data together indicated that spadin has no side effects either on TREK-1 controlled functions or at the cardiac level. Spadin reversed depressive state in naïve mice and also in depressed mice. Spadin can be considered as a putative endogenous antidepressant of new generation with a rapid onset of action. Its efficacy will be improved by the identification of more potent analogs.

Oral presentation

Duct-lining cells can reprogram into beta-like cells able to counter repeated cycles of toxin-induced diabetes.

PFEIFER Anja[123], AL-HASANI Keith[1234], COURTNEY Monica[123], BEN-OTHMAN Nouha[123], GJERNES Elisabet[123], VIEIRA Andhira[123], DRUELLE Noemie[123], AVOLIO Fabio[123], COLLOMBAT Patrick[123]

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2: Université de Nice-Sophia Antipolis, FR-06108 Nice, France

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4: Baker IDI Heart and Diabetes Institute, The Alfred Medical Research and Education Precinct, Melbourne, Victoria, 3004, Australia.

Keywords: type i diabetes, pancreas, pax4, mouse models, reprogramming, endocrine pancreas development

Background and aims Diabetes mellitus, which is the result of a cell-mediated autoimmune destruction of beta-cells, has become one of the most widespread metabolic diseases, affecting almost 6% of the world's population. Although insulin-based therapy can provide a measure of control of the glycaemia in T1DM patients, exogenous insulin can still not ensure physiologically stable blood glucose levels. Differences in diet, age and/or exercise can indeed cause significant variations in the glycaemia. Such fluctuations represent a major burden, since they can, over time, lead to a wide range of complications, including micro- and macro-vascular damages, blindness, amputation and/or death. Alternative therapies, such as islet transplantation or in vitro generation of allogenic beta-cells, present a number of disadvantages, including life-long immunosuppression, the scarcity of cadaveric donors, or technical inefficiencies. Methods and results Using the mouse as a model, it was recently demonstrated that immature glucagon-producing cells in the pancreas can regenerate and become converted into insulin-producing beta-like cells through the constitutive/ectopic expression of the transcription factor Pax4. However, it remains unclear whether glucagon+ alpha-cells in adult mice display the same plasticity. Similarly, the mechanisms underlying such reprogramming are still poorly understood. Taking advantage of an inducible transgenic mouse model that allows us to misexpress Pax4 in glucagon+ cells age-independently we demonstrate now, that the misexpression of Pax4 promotes alpha- to beta-like cell conversion and their glucagon shortage-mediated replacement, resulting in islet hypertrophy and in an unexpected islet neogenesis in adult mice. Taking advantage of several lineage-tracing approaches, we show that, upon Pax4-mediated alpha-to-beta-like cell conversion, pancreatic duct-lining precursor cells are mobilized, re-express the developmental gene Ngn3, and successively adopt a glucagon+ and a beta-like cell identity through a mechanism involving the reawakening of the epithelial-to-mesenchymal transition. Conclusion The processes induced upon Pax4 misexpression in adult alpha-cells can repeatedly regenerate the entire beta-cell mass, and thereby reverse several rounds of chemically-induced diabetes, thereby providing promising perspectives to design novel therapeutic regenerative strategies.

Oral presentation

The deubiquitinating enzyme USP14 controls MCL-1 expression and leukemic cell survival.

DUBOIS Nicholas[1], MALLAVIALLE Aude[1], DELMONT Emilien[12], MAXENCE Garel[1], JEANDEL Pierre-yves[1], TICCHIONI Michel[12], TARTARE-DECKERT Sophie[1], DECKERT Marcel[1]

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2: CHU Nice, France

Keywords: chronic lymphocytic leukemia, deubiquitinating enzymes, usp14, mcl-1, syk

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western countries and represents approximately 40% of leukemia in individuals over 65 years. The evolution of this disease is very heterogeneous because some patients die within the first two years after diagnosis, while others survive for more than 20 years without treatment. But despite recent therapeutic advances, CLL remains largely incurable. CLL is characterized by immune dysfunction and severe hematological complications due to the accumulation of leukemic B cells. Pathological cells show reduced capacity to be eliminated by apoptosis. This resistance is the result of the abnormal overexpression of apoptosis inhibitory proteins, such as MCL-1 protein, an anti-apoptotic BCL-2 family member. Overexpression of anti-apoptotic proteins such as MCL-1 is also responsible for resistance to current chemotherapies. This overexpression is the result of genetic and epigenetic alterations, dysregulation of cell survival pathways and signals from the tumor microenvironment. MCL-1 is a protein with a very short half-life, resulting from its rapid degradation by the ubiquitin-proteasome system following its phosphorylation by GSK3-beta and its ubiquitination by E3 ubiquitin ligases such as beta-TRCP or FBXW7. Our team has demonstrated the importance of the signaling pathways controled by the protein tyrosine kinase Syk in the prevention of MCL-1 degradation. However, the nature of the deubiquitinating enzyme(s) (DUBs) that may regulate MCL-1 expression is still unclear. Using a HA-tagged biochemical probe that covalently reacts with active DUBs from protein cell extracts, our preliminary observations suggest an involvement of DUBs in the survival of CLL cells. Using new pharmacological agent inhibitors of DUBs that show cytotoxic properties on leukemic cells, we identified a panel of DUBs potentially involved in resistance to apoptosis and decreased expression of MCL-1. Among them, the ubiquitin specific protease (USP) 9X (USP9X) and 14 (USP14) were identified as the two major active DUBs in leukemic cells. In contrast to a recent report, we could not find any evidence that USP9X regulates the levels of MCL-1 in leukemic cells. In contrast, inhibition of USP14 by pharmacological agents and its extinction by siRNA reduced Mcl-1 expression and cell survival. In addition overexpression of USP14 increased MCL-1 level. Very little is known about the regulation of DUBs activity. The biochemical HA-Ubiquitin probe allow us to observe the active form of DUBs and for the first time, by pharmacological approch, we show that USP14 DUB activity is regulated by survival signals transmitted by the tyrosine kinase Syk through the activation of the kinase AKT. Our study indicates that the proteasome-associated USP14 deubiquitinating enzyme represents a novel key regulator of the antiapoptotic protein MCL-1 and underlines the interest of certain DUBs as potential therapeutic targets in CLL treatment.

Oral presentation

The chinese medicine neuroaid (mlc601, mlc901) induces potent neuroprotective and neuroproliferative effects after global cerebral ischemia and traumatic brain injury in rodents.

QUINTARD Herve[1], GANDIN Carine[1], LORIVEL Thomas[1], LAZDUNSKI Michel[1], HEURTEAUX Catherine[1],

1: Institut de Pharmacologie Moléculaire et Cellulaire, UMR7275

Keywords: mlc601, mlc901, brain trauma, global cerebral ischemia

Introduction Cardiac arrest or brain trauma are responsible for important disabilities. Mechanisms involved in brain injury are now well described as apoptosis, necrosis, inflammation and oxidative stress following by a neurorestorative time as neurogenesis. NeuroAid (MLC601 and MLC901), a Traditional Chinese Medicine, which is used in China for patients after stroke was recently described to be protective in models of focal ischemia. Our aim was to investigate the effectiveness of MLC901 on brain injury and deficits after global cerebral ischemia and brain trauma induced by a lateral fluid percussion in rodent. Methods All experiments were performed on male(250 g) Sprague Dawley rats (trauma)and Wistar (ischemia)from Charles River Laboratories (France) and used according to policies on the care and use of laboratory animals of European Communities Council Directive (86/609/EEC). The local Ethics Committee approved the experiments. Traumatic brain injury was induced by Lateral Fluid Percussion (LFP) (AmScien Instruments, Richmond, VA, USA) (2 Atm). Ischemia was conducted according Pulsinelli technique. The study was carried out on rats divided into three groups: Sham , trauma /ischemia vehicle or MLC901 (Moleac (Singapore) injected with a single IV dose (500 ?l/rat) followed by enteral feeding. Importance of brain lesion explored by histologic coloration (ttc, cresyl violet, Fushine coloration), MRI , PS100B , apoptosis, oxydative stress and brain edema quantification. .Neurorestorative phasis investigated by BrDU staining, Fluorogold technique , and growth factors (BDNF and VEGF)staining. Comportment was also investigated (water maze, actimetry, grip strength, object recognition) RESULTS In the two models, we described important brain injury after ischemia and trauma. MLC limits these injuries by different ways (decrease in lesion volume, limits edema, loss of neurons in CA1, apoptosis and oxydative stress lesions). Neurorestorative mechanisms are enhanced by treatment (increase in imature cells in Dentate gyrus). It conducts to an improvement in functional recovery in all tests realised. CONCLUSION In an ischemic and a traumatic models, MLC has neuroprotective and neurorestorative effect associated with an improvement in functional recovery.

Oral presentation

Study of the role of the AR-JP associated protein parkin in the development of human brain tumors.

VIOTTI Julien[1], DUPLAN Eric[1], CAILLAVA Céline[1], CONDAT Joris[1], GOIRAN Thomas[1], GIORDANO Cécile[1], MARIE Yanick[23], IDBAIH Ahmed[23], DELATTRE Jean-yves[23], HONNORAT Jérôme[4], CHECLER Frédéric[1], ALVES DA COSTA Cristine[1]

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Keywords: glioma, p53, parkin, mutations

Glioma represents the most frequent form of brain tumor, the prognosis of which remains extremely poor. The etiology of gliomas is tightly linked to an age-related increase of the rate of p53 somatic mutations that ultimately leads to its inactivation. Parkin is a tumor suppressor protein, the mutations of which have been linked to glioblastoma development. We have shown that parkin could control apoptosis via the down-regulation of p53 transcription. We have thus decided to examine the interplay between parkin and p53 in glioma genesis. Q-PCR, Western blots and viral infection techniques were used to assess the regulation of mRNA and protein of parkin by p53, ex-vivo and in vivo. DNA binding p53-dead mutations effect on parkin regulation was also examined. We show that parkin levels inversely correlate to brain tumor grade and p53 levels in oligodendrogliomas, mixed gliomas and glioblastomas. Furthermore, distinct cells models invalidated for p53 gene or harboring transcriptionally inactive p53 show decreased parkin promoter activity, mRNA and protein levels. Importantly these results were validated in vivo by overexpressing p53 in the mice brain. Finally, we demonstrate that p53 inactivating mutations abolish p53 ability to transactivate parkin confirming that p53 controls parkin via its DNA binding properties. In conclusion, our work delineates a functional interplay between mutated p53 and parkin in glioma genesis that is disrupted by cancer-linked pathogenic mutations. It also allows envisioning parkin as a novel biomarker of glioma progression.

Oral presentation

Purification and characterization of ribonucleoprotein particles associated with the L1 ORF1p protein.

VIOLLET Sébastien[123], PISANO Sabrina[1], CRISTOFARI Gaël[1]

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2: Inserm U1081
3: Faculty of Medicine

Keywords: genome plasticity, retrotransposition, ribonucleoprotein, line-1, atomic force microscopy

The Long Interspersed Nuclear Elements 1 (LINE-1 or L1) are abundant retrotransposons in the human genome which can proliferate through a copy and paste mechanism. Active L1 elements code for two proteins: a 40 kDa trimeric RNA binding protein named ORF1p, and a large protein, ORF2p, which exhibits endonuclease and reverse transcriptase activities. These proteins assemble in cis with their own encoding mRNA to form a stable ribonucleoprotein particle (L1 RNP), which is the core of the L1 retrotransposition machinery. After its assembly, it is imported into the nucleus, where new L1 copies will be synthesized and integrated at new genomic DNA loci, through a coordinated mechanism called target-primed reverse transcription (TPRT). To get further insights into the composition and biogenesis of L1 RNPs, we fused an epitope tag to the ORF1p protein in a plasmid-borne replication-competent L1 element. After expressing this tagged-L1 in human cells, we applied affinity chromatography to purify soluble native L1 complexes assembled in vivo. We confirmed that L1 RNA and ORF2p co-purify with ORF1p. By combining this new tool and the atomic force microscopy, we show for the first time ORF1p complexes and underline a new mechanism for the assembly of ORF1p trimer unrelated to the cis-preference.

Oral presentation

Variability and evolution of the venom of two biological control agents from the Psyttalia genus.

MATHÉ-HUBERT Hugo[1], COLINET Dominique[1], BELGHAZI Maya[1], THAON Marcel[1], GATTI Jean-luc[1], RIS Nicolas[1], MALAUSA Thibaut[1], POIRIÉ Marylène[1]

1: UMR Institut Sophia Agrobiotech, INRA 1355, CNRS 7254

Keywords: parasitoid, venom, biological control, variability, evolution

Endoparasitoid wasps, largely used as biological control agents, are insects that develop at the expense of their insect host, leading to its death. Their reproductive success largely depends on their capacity to control the host physiology and immunity (Dupas et al. 2003). Parasitoids have thus evolved highly diversified virulence strategies to achieve parasitism success but most of them rely on the injection of venom proteins along with the eggs. Previous studies have shown that venom is very diversified even between closely-related species (Crawford et al. 2008; Colinet et al. 2012). The intra-specific variability have also been shown to be surprisingly high even between individuals (Colinet et al. 2012). This high variability suggests that the evolutionary potential of venom may be high and that venom components thus likely vary among populations of candidate biological control agents. In this work, we have used some P. lounsburyi sampled in the field in South Africa and Kenya to follow the evolutionary response of the venom to the laboratory conditions like the host shift from the natural host B. oleae to the substitution host C. capitata. The venom content has also been characterized by a combined transcriptomic and proteomic approach in order to identify the putative function of venom's proteins and explain the observed variation and evolution in venom contents. This work provides encouraging results about the relevance of venom to characterize and monitor biological control agents and to study the intra-specific variability of trophic interactions.

Oral presentation

Study of the interaction between FMRP and GRK mRNA.

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Keywords: fmrp, grk4, fxs, rna binding

Fragile X syndrome (FXS) is the most common form of inherited mental retardation and is caused by the silencing of the FMR1 gene, which encodes for an RNA-binding protein (FMRP) moslty involved in translational control. We characterized here in vivo and in vitro the interaction between FMRP and the mRNA of GRK4. This gene encodes a member of the guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily. FMRP negatively modulates the expression of GRK4 at the translational level only in cerebellum, suggesting the co-regulation with cerebellum-specific factors. We precisely defined a portion of the coding region of the GRK4 mRNA containing 6 ACUK/WGGA motif repetitions, known to represent novel targets for FMRP (Ascano et al., 2012) . Intriguingly, in our analysis, FMRP binds this target via its RGG box domain, and not through its KH2 domain, as other authors proposed. In general, GRK4 phosphorylates the activated forms of G protein-coupled receptors, thus initiating their deactivation. However, it has been shown that, in cerebellar granule cells, GRK4 interacts directely with the GabaB receptor (GBR), promoting its desentitization that occurs in the absence of an increased receptor phosphorylation and does not require the catalytic activity of the kinase (Perroy et al., 2003). Since in cerebellum GBRs signalling has a relevant role in motor coordination, and elevated level of GRK4 can determine a desensitization of GBR specifically for this brain region and contribute to deficits of motor learning and movement coordination that are cerebellum-dependent phenotypes of FXS.

Oral presentation

Osteoarthritis: from epidemiology to fundamental: Chondrogenic potential of stem cells derived from adipose tissue and ocytocine action on chondrocyte.

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Keywords: chondrocyte, osteoarthritis, differeciation, clinical, epidemiology

Introduction: Osteoarthritis is the most frequent joint disease in the world, its frequency will increase in the future with the expected aging of western populations. Our work has led us to explore clinical, therapeutical, and mainly epidemiological aspects of osteoarthritis which were missing in France on one hand and to characterize the chondrogenic potential of the hMADS cells. Material and methods hMADS cells and BM-MSC were seeded at a density of 4500 cells/cm2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 2.5 ng/ml hFGF2. The medium was changed every other day and hFGF2 was removed when cells reached confluence and were triggered for differentiation at day 2 post-confluence (designated as day 0). Cells were maintained in differentiation medium containing TGF ß3 in the absence or the presence of BMP6. The expression of type-I and type-X collagens, Aggrecan and COMP was estimated by RT-qPCR. Matrix components were revealed using Alcyan blue, safranin and toluidine blue stainings as well as immuno-histochemistry. The effect of IL-1, its antagonist as well as of nicotine on the expression of different matrix component genes was investigated. Results: We showed that hMADS cells were able to synthesize matrix proteins including COMP and Aggrecan, with a gradual increase throughout the chondrogenesis process. BMP6 treatment inhibits clearly the increased expression of Col X (4-fold decrease) compared to a medium without BMP6. Staining performed at different time points of chondrogenesis highlighted a gradual increase of the extra-cellular matrix proteins. These data were confirmed by immunostaining. In addition, the cells expressed BMP receptors with a temporal evolution similar to that shown in the literature. Altogether these observations confirmed the chondrogenic capacity of hMADS cells. Then, we tested whether IL-1 and nicotine could impact chondrocyte differentiation. As expected, IL-1 affected negatively chondrogenesis which was reverted in the presence of IL-1 antagonist and these effects were mediated through ADAMTS4 and ADAMTS5. The effects of nicotine on chondrocyte differentiation are not well documented and unclear. Nicotine exerted a slight positive dose dependent effect suggesting a protective effect of nicotine at a concentration close to the average nicotine concentrations of smokers (25 µg/ml) and higher concentrations were associated with an important increase of Col X. Conclusion: hMADS cells are able to differentiate into chondrocytes and respond to different pharmacological stimuli. Therefore, these cells represent a valuable tool for the analysis of in vitro chondrocyte differentiation and the test of potentially interesting pharmacological drugs. Analysis of the effect of oxytocin on the chondrocyte formation in vitro using hMADS cells and in vivo using an animal model is underway and will be completed in the next coming months.

Oral presentation

Inducible misexpression of Ngn3 in pancreatic duct-lining cells.

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Keywords: diabetes, pancreatic development, mice, neurogenin3, insulin, duct

Background and aims: In the search for improved therapies for type 1 diabetes, a large amount of research has involved replenishing the beta-cell mass from cells already present within the pancreas whether it be in the exocrine or endocrine compartment. We have previously shown that the misexpression of Pax4 in glucagon-expressing/alpha-cells leads to their conversion into functional insulin-expressing cells displaying most features of true beta-cells. This conversion was accompanied by increased proliferation in the ductal epithelium and lining and a reactivation of the proendocrine marker Ngn3. These results led to the hypothesis that cells within the ductal epithelium re-express Ngn3, differentiate into glucagon-expressing cells and are subsequently converted into beta-like cells. Thus, the objective of the current project is to investigate the potential of cells from the ductal epithelium/lining to differentiate into endocrine cells by the sole forced expression of Ngn3. Methods: In order to specifically target duct cells within the pancreas, Hnf1? -CreER animals were used as HNF1? is solely expressed in duct cells of the mouse pancreas. This line was then crossed with a mouse line allowing the Cre-mediated expression of Ngn3 in duct cells upon tamoxifen treatment. The resulting animals are healthy, show no developmental issues or gain of weight and display normal basal glycaemia. In order to optimize the induction of Ngn3 in this system, different methods of tamoxifen administration and different times of treatment have been tested. Results: After short time treatment by gavage in these animals, our preliminary results indicate modest islet hyperplasia. Monitoring endocrine cell contents in these islets and in those of animals treated for longer time, this hyperplasia seems to be the result of an increase in all endocrine cells type, consistent with the role of Ngn3 in pancreatic development. Further characterization of these animals is ongoing at the level of islet architecture as well as at the functional level. These ongoing experiments will be presented. Conclusion: This ongoing work will help us determine whether ductal cells within the pancreas have the ability to differentiate into endocrine cells upon the sole activation of Ngn3.

Oral presentation

Structural model of the anion exchanger 1: idenfication of the transport site.

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Keywords: anion exchanger 1, membrane protein, cystein scanning

The Anion Exchanger 1 (AE1), member of bicarbonate transporter family SLC4, is one of the most abundant proteins of the vertebrate red cell membrane. AE1 mediates an electroneutral one to one chloride versus bicarbonate exchange in physiological conditions In red cells it participates to crucial different tasks including: CO2 transport, intracellular pH regulation, and has a structural role by anchoring the ankirin/spectrin cytoskeleton to the plasma membrane. Some point mutations in AE1 membrane spanning domain convert the electroneutral anion exchanger into a Na+ and K+ conductance or induce a cation leak in a still functional anion exchanger. The molecular determinants that govern ion movement through this transporter are still unknown. The present work was intended to identify the ion translocation pathway within AE1. Without a sufficient resolutive three-dimensional structure of AE1 membrane spanning domain, a combined approach with in silico study, site-directed mutagenesis and structure/function analysis have been done A structural model of AE1 membrane spanning domain is proposed for the first time and this model is based on the structure of Uracil-proton symporter. This model was used to design cysteine-scanning mutagenesis on transmembrane segments (TM) 3 and TM5. By measuring AE1 anion exchange activity or cation leak it is proposed that there is a unique transport site comprising TM3-5 and TM8 that should function as an anion exchanger and a cation leak This work will facilitate identifying and studying other key residues and TMs in wild-type AE1 and pathological mutants. Moreover, it could help analysis of future high definition crystals of the spanning domain of AE1 and support other studies of AE1 in vitro and in vivo.

Oral presentation

Occurrence of metastasis and in vitro aggressiveness of triple negative breast cancer is related to the glycolytic phenotype, microenvironment metabolism and MCT4 lactic acid transporter.

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Keywords: breast cancer, glycolytic metabolism, prognostic factors, metastasis

Background: Breast cancer is divided in three subgroups: hormone-receptor positive (70%), Her-2 subgroup (15%) and the triple negative (TNEG) subgroup (15%) where hormone receptors and Her-2 are lacking. Because TNEG breast cancers does not respond to hormone-therapy or anti-Her-2 therapy intense research is ongoing to find new target in this subgroup which has the worst prognosis. Comparing with hormone positive and Her-2 overexpressing breast cancers, TNEG breast cancers are characterized by a more systematic and far stronger 18-FDG uptake that suggests a glycolytic addiction in this tumour subset (PMID: 18098228 and 22011459). Material and methods: Correlation between proteins involved in glycolytic metabolism and intracellular pH regulation (c-myc, GLUT-1, LDH-A, MCT1, MCT4, Basigin, CA9) and metastasis has been analyzed in 158 patients with TNEG disease using tissue-micro arrays. Proteins that were indicative of poor prognosis were inhibited or knocked-out in vitro to determine potential new targets in TNEG breast cancers. Results: With a median follow-up of 6 years (0.25-14.4) the metastasis rate was 27.4% at 6-years. Tumours were highly proliferative (70% with Ki-67 staining > 20%) and had a glycolytic phenotype with a strong expression of GLUT-1 (78%), LDH-A (78%), MCT1 (60.4%), MCT4 (85.7%), Basigin (78%), CA9 (53.7%) and C-myc (84.2%). The Log-rank test identified as bad prognostic factors for metastasis-free survival (MFS), large pT ($p<0.001$), positive pN ($p=0.001$), positive MCT4 ($p=0.01$) and LDH-A ($p=0.04$). MCT4 staining and LDH-A staining were even stronger prognostic factors when excluding tumors with stromal staining ($p=0.001$ and $p=0.005$ respectively for MFS). Ki-67, age and grade were not correlated with MFS. pT stage, pN and tumoral MCT4 staining were considered as independent prognostic factors in multivariate analysis (Cox regression, HR of respectively 2.4, 2.2 and 2.2, $p<0.05$). These results were similar for overall and cancer specific survival. In vitro, inactivation of MCT4 in a TNEG cell line (HS578t) reduced clonogenic survival by 50%. Use of the strong inhibitor of the mitochondria respiratory chain, phenformin, resulted in a reduction of clonogenic survival. The highest reduction of clonogenic survival was observed when combining phenformin with MCT1/2 inhibitor. Conclusions: This study demonstrates the role of glycolytic metabolism in the tumour microenvironment and in the occurrence of metastases of TNEG breast cancer. Our in vitro data suggests that targeting this metabolic cancer hallmark in combination with phenformin could provide a potential novel anticancer treatment for TNEGs.

Oral presentation

METABOLISM AND CANCER: EXPLORATION OF A POTENTIAL METABOLIC CORPERATION BETWEEN TUMOR ASSOCIATED MACROPHAGES AND CARCINOMA CELLS.

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Keywords: cancer, metabolism, tumor associated macrophages

Tumor cells mainly rely on glycolysis for energy production and yet, their tumor microenvironment (stroma) supplies insufficient amount of nutrients. We therefore hypothesize that it exists a nutritional symbiosis between stromal cells and tumor cells which contribute to tumorigenesis. Of these stromal cells, we focused on Tumor Associated Macrophages (TAMs) and their potential metabolic cooperation with carcinoma cells. Typically, macrophages are classified as inflammatory (M1-like) or alternative (M2-like) and TAMs are mainly considered as M2-like macrophages. Concerning their metabolic profile, M1-like macrophages have been described to exhibit a glycolytic metabolism. (Vats D. et al., Cell Metab., 4(1), 2006 , Rodriguez JC et al, J Immuno, 2010). However, the metabolic features of M2-like macrophages remain pretty unclear. Our project is to characterize the metabolic requirements of M2-like versus M1-like macrophages exposed to tumor microenvironment conditions (such as hypoxic conditions, low glucose or lactate-rich medium) and to determine the contribution of macrophage metabolism to carcinoma cell behavior. In parallel, we study how cell metabolism could impact on macrophage polarization. First, using a macrophage established cell line (RAW 264.7), we characterized the metabolic features of M1 and M2-like macrophages by measuring both glycolysis and respiration. We confirmed that in normoxic conditions, M1-like macrophages are exclusively glycolytic. We showed that M2-like macrophages display a more oxidative profile although they are able to switch to glycolysis when respiration is blocked. Under either a low glucose concentration (0.1mM) or hypoxic conditions, M2-like macrophages lose their ability to switch to glycolysis when respiration is blocked. For M1-like macrophages, no change is observed under a low glucose concentration but they tend to be more glycolytic under hypoxic conditions. Finally, we observed that lactate is a less efficient substrate than glucose for both M1 and M2-like macrophages. From these results, glycolysis seems to play a major role in macrophage metabolism when they are placed under conditions that mimic the tumor microenvironment. As a result, we have blocked the glycolytic pathway of these macrophages by simultaneously knocking-out the lactate/H+ hypoxia-inducible symporter MCT4 in RAW 264.7 cells by the zinc finger nuclease technique and inactivating the lactate/H+ symporter MCT1 using a specific pharmacological inhibitor (Le Floch et al, PNAS, 2011). MCT4-/- cells have been obtained and characterized. When both MCT4 and MCT1 are blocked under normoxic conditions, M1-like macrophages exhibit a decrease in their ability to perform glycolysis and show a slight increase in their ability to do oxidative phosphorylation. However, M2-like macrophages lose their ability to switch to glycolysis when respiration is blocked. We are now evaluating the effect of blocking the export of lactic acid on the proliferation, migration and invasion of carcinoma cells. In parallel, we demonstrated that macrophage metabolism could impact on its own polarization. Actually, when the glycolytic pathway is impaired (using the MCT4-/- cells treated with the MCT1 inhibitor), the M1-like macrophages tend to switch to an M2-like phenotype. And M2-like macrophages have their M2 phenotype strengthened. A deeper characterization of this effect is required to better understand how metabolism could control macrophage polarization.

Oral presentation

Metformin Blocks Melanoma Invasion and Metastasis Development in AMPK/p53-Dependent Manner.

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Keywords: metformin, melanoma

Metformin was reported to inhibit the proliferation of many cancer cells, including melanoma cells. In this report, we investigated the effect of metformin on melanoma invasion and metastasis development. Using different in vitro approaches, we found that metformin inhibits cell invasion without affecting cell migration and independently of antiproliferation action. This inhibition is correlated with modulation of expression of proteins involved in epithelial-mesenchymal transition such as Slug, Snail, SPARC, fibronectin, and N-cadherin and with inhibition of MMP-2 and MMP-9 activation. Furthermore, our data indicate that this process is dependent on activation of AMPK and tumor suppressor protein p53. Finally, we showed that metformin inhibits melanoma metastasis development in mice using extravasation and metastasis models. The presented data reinforce the fact that metformin might be a good candidate for clinical trial in melanoma treatment.

Oral presentation

Implication of the inflammatory kinase Tpl2 in the deleterious effects of cytokines and macrophages on adipocytes functions.

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Keywords: adipocyte, macrophage, tpl2, insulin, cytokines

Adipose tissue (AT) inflammation and dysfunction are involved in the development of the complications of obesity. In obesity, AT is infiltrated by macrophages that contribute to the production of cytokines. These cytokines alter the functions of adipocytes leading to a decrease in insulin signaling and action and to an increase in lipolysis. Further, free fatty acids produced by dysfunctional adipocytes have an inflammatory effect on macrophages. We have recently reported that the MAP3 kinase Tpl2 was a new inflammatory kinase involved in the deleterious effect of cytokines on adipocytes function. The aim of the present study was to determine the implication of Tpl2 in the cross-talk between macrophages and adipocytes and in the deleterious effect of macrophages on adipocytes function. We found Tpl2 expression was up-regulated in AT of obese mice and patients both in adipocytes and in pro-inflammatory CD11c+ cells (macrophages). A co-culture between adipocytes and macrophages enhanced the production of inflammatory cytokines and the lipolysis and decreased insulin signaling in adipocytes. Pharmacological inhibition of Tpl2 in the co-culture markedly suppressed the production of cytokines and free fatty acids and partly restored the insulin signaling in adipocytes. Invalidation of Tpl2 in macrophages markedly reduced the cytokines expression in the co-culture system. A conditioned medium of macrophages treated with low dose of LPS inhibited insulin signaling in adipocytes. Treatment of macrophages with a Tpl2 inhibitor or siRNA markedly reduced the deleterious effect of the conditioned medium on insulin signaling and action in adipocytes. We conclude that Tpl2 pathway may constitute a mediator in the cross-talk between adipocytes and macrophages in adipose tissue leading to sustained inflammation. Inhibition of this pathway may reduce the production of inflammatory cytokines by the macrophages and improved adipocytes insulin signaling and functions.

Oral presentation

Sigma-1 receptor (Sig1R) regulates tumor / extracellular matrix interactions through modeling electrical plasticity – consequences on cancer progression.

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Keywords: sigma-1 receptor, leukemia, extracellular matrix, ion channels, herg

Understanding mechanisms regulating the tumor cells / microenvironment cross talk is crucial challenge to propose new therapeutic target against cancer progression. Ion channels are often aberrantly expressed in tumor cells and represent an under-estimated source of targets. Recently, the cardiac potassium channel hERG (human ether-a-go-go related gene) was demonstrated involved in the cross talk occurring between leukemia cells and the extracellular matrix (ECM) through a close interaction with active Bêta-1 integrin. Despite its promising potential as a therapeutic target, direct inhibition of hERG may have important issues on cardiac activity. Sigma-1 receptor (Sig1R) is an ER-localized chaperone protein over-expressed in many cancers. Sig1R regulates calcium homeostasis during ER stress and ion channels represent a predominant class of clients for its chaperone function. Previously, we demonstrated in leukemia cells that Sig1R regulates hERG trafficking by promoting channel maturation. However, the functional significance of Sig1R/hERG interaction in cancer is unknown. Herein, we demonstrate that leukemia / ECM interactions induce a Sig1R-dependent remodeling of cellular electrical activity. Indeed, in response to ECM-induction Sig1R promotes a fast mobilization of hERG channels, stimulating the formation of hERG / Bêta-1 integrin complexes at the plasma membrane and the subsequent activation of Akt signaling pathway and the growth factor secretion. Finally, we observed that Sig1R regulates migration, invasion, extravasation and tumor-associated angiogenesis processes in vitro and in vivo. Thus, we demonstrate that Sig1R potentiates cancer progression by regulating ECM-dependent tumour cell plasticity. Sig1R may thus represent a very promising therapeutic target to alter the tumor / micro-environment cross talk and prevent tumor progression.

Oral presentation

A novel molecular mechanism specifying different subpopulations of layer V neurons projecting to the pons or spinal chord in the developing mouse cortex.

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Keywords: mouse cortex, coup-tf1, lmo4, satb2, ctip2, bhlhbb5, somatosensory area, layer v, spinal cord, pons

The mammalian neocortex is organized into six layers, in which neuronal populations can express different neuronal subtype-specific genes and have distinct morphology and connectivity patterns. For example, layer V neurons consist of different types of projection neurons (PNs): corticofugal, including subcerebral and corticostriatal PNs, and corticocortical, including callosal PNs. Fezf2, Satb2 and Ctip2, known to regulate each other, are involved in the specification of layer V PNs during development. While Fezf2 promotes the expression of Ctip2 and the specification of subcerebral PNs, while Satb2 is a determinant gene for callosal identity. Moreover, in layer V Fezf2 inhibits Satb2, which, in turn, represses Ctip2 transcription by recruiting HDAC1 and MTA2 (members of the NURD complex) to the Ctip2 locus. However, in P0 brains, we found that a small population of layer V neurons still expresses high levels of Satb2 and Ctip2. In the absence of COUP-TFI, described by our group to be a key transcriptional regulator during cortical areal and cell-type specification (1, 2), the number of double Satb2/Ctip2-expressing cells in layer V increases abnormally in the prospective somatosensory area S1. We also found that LMO4, normally expressed in corticocortical PN of frontal and occipital regions of P0 brains, is ectopically expressed also in the parietal ones and suppresses the inhibition of Ctip2 by Satb2 by binding to HDAC1 and interfering with the formation of the NURD complex. Our analysis showed that the number of Satb2/Ctip2-expressing cells increases with time in WT conditions from p0 to p7 to P21, together with the increase of LMO4 during these stages, and so this population is anticipated since p0 in the absence of COUP-TF1 due to the earlier increase in LMO4 expression. Fluorogold Injections show that this cell population is divided into 2 different sub-populations that project to the spinal cord or the pons, depending whether they molecularly express LMO4 or Bhlhb5. DiI injections show that this cell population has collaterals to the striatum. An altered balance between these 2 sub-populations is shown in the absence of COUP-TF1. Using a transgenic mice line, labeling layer V neurons we are characterizing the morphology and neuron ramifications of these different sub-populations. Together, our results unravel a novel molecular mechanism specifying a cortical PN population within layer V.

Oral presentation

Production of LIF cytokine by cancer cells and fibroblasts contributes to the establishment of a pro-invasive tumor microenvironment.

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Keywords: tumor microenvironment, carcinoma associated fibroblasts, matrix remodelling, cancer cell invasion.

Tumor-stroma signaling crosstalk contributes to tumor microenvironment modifications and cancer cell spreading. Carcinoma associated fibroblasts (CAF) display enhanced extracellular matrix remodeling capacities and formation of a pro-invasive tumor microenvironment, which enables carcinoma cell collective invasion. The TGF-beta cytokine-dependent signaling pathway was considered the major CAF activator. Using three-dimensional organotypic invasion assays, we investigated the molecular mechanisms of the TGF-beta1-dependent signaling that mediates the pro-invasive fibroblast activation. We demonstrate that TGF-beta induced LIF production by both cancer cells and fibroblasts is responsible for the pro-invasive tumor microenvironment modifications. LIF cytokine stimulation of human dermal fibroblasts activates pro-invasive track formation in a GP130/JAK1/STAT3 specific dependent signaling pathway. We show that LIF mediates TGF-beta-dependent acto-myosin contractility leading to collagen fiber assembly. In subsequent screenings, 11 out of 12 human carcinoma cell lines from different organs (skin, head and neck, breast and colon) and 2 out of 3 human melanoma cell lines induce pro-invasive fibroblast activation in vitro through direct secretion of LIF. Moreover, detection of LIF cytokine in human skin SCC biopsies indicated that LIF is significantly up-regulated in tumor tissues. Finally, an orthotopic mice model of breast carcinoma demonstrates that LIF production correlates with tumor microenvironment collagen fiber organization and cancer cell invasion in vivo. These results disclose the molecular mechanisms underlying the pro-invasive activation of stromal fibroblasts in tumor contexts and identify LIF cytokine as a key player in the process. They also suggest that blocking JAK1 kinase expression in CAF may present potential therapeutic benefits for patients with aggressive carcinoma.

Oral presentation

The role of the nuclear receptors COUP-TFs in the migration and the specification of cortical interneurons during mouse development.

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Keywords: interneurons, coup-tfi, coup-tfii, migration, specification

In rodents, cortical interneurons originate from the medial ganglionic eminence (MGE) and caudal ganglionic eminence (CGE) according to precise temporal schedules and reach their final laminar position through tangential and radial cell migration. Although great progress has been made over the last decade in elucidating the molecular diversity and fate-specification of MGE-derived interneuron subtypes, the mechanisms controlling the migration and specification of CGE-derived interneurons are still unknown. The project is based in challenging the function of two orphan nuclear receptors, COUP-TFI and COUP-TFII, which are broadly and specifically expressed within the CGE and required for the migration and specification of interneurons. To study the migration and the specification of these CGE-derived interneurons, COUP-TFI and COUP-TFII were conditionally inactivated in all GABAergic interneurons that arise from the subventricular zone of the ventral telencephalon. My previous data have shown that conditional loss-of-function of COUP-TFI in precursors and post-mitotic interneurons leads to cell migratory defects and to a decrease of late-born, CGE-derived interneurons, within the cortex. This defect is compensated by a concurrent increase of early-born, MGE-derived interneurons, ultimately resulting in altered cortical activity. I also found that the COUP-TFI homologue, COUP-TFII, is abnormally highly expressed in the absence of COUP-TFI, leading to the hypothesis that both members of the same subfamily might contribute to the migration and specification of cortical interneurons. My recent results indicate that only a subpopulation of interneuron precursors expresses both COUP-TF genes at early developmental stages and that interneuron subtypes are differently affected in both mutants. Although the total number of GABAergic interneurons is not altered, their migratory pattern and subtype distributions in the mature cortex seem to be differently affected, with the exception of the calretinin-expressing population, which is diminished in both COUP-TF mutant mice. I am at present generating double COUP-TFI and COUP-TFII conditional mice and expect that invalidation of both members should lead to a stronger phenotype than just invalidating one gene. To characterise the interneuron migratory defect and identify molecules required for cell migration, I have crossed the COUP-TF conditional mouse lines with the CGE-specific reporter line, 5HT3aR-GFP. Careful analysis in a normal brain highlighted a novel, previously non-described, caudo-rostral migratory route from the CGE to the MGE and from the CGE to the lateral ganglionic eminence (LGE). This caudo-rostral tangential cell migration might be crucial for understanding the laminar organization of interneurons during corticogenesis. At present, I found that both COUP-TF genes control the proportion of these two streams in a dosage-dependent manner. I also found that distribution of the receptor Neuropilin2 is affected in COUP-TF mutant embryos, indicating that alteration of the neuropilin-semaphorin pathway might explain the altered migratory path. Finally, the radial migration of interneurons within the cortex, essential for the final laminar-specific distribution of interneuron subtypes in the mature cerebral cortex, is also perturbed in the absence of COUP-TF genes. In summary, my data suggest an important role of COUP-TFs in the specification and migration of late-born cortical interneuron subtypes.

Oral presentation

Functional analysis of the RSPO1/beta-catenin signaling pathway in normal ovarian development and in ovarian pathologies.

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Keywords: ovary, folliculogenesis, rspo1, beta-catenin pathway, mouse models

Ovarian cancers represent the second cause of death by gynecological tumor after breast cancer. Implication of the beta-catenin signaling pathway in the etiology of some ovarian cancers has already been shown. However, the early steps leading to the formation of these tumors remain to be identified. R-spondin1 is an activator of the beta-catenin signaling pathway which is important for the ovarian folliculogenesis occuring after differentiation of the ovary. During my PhD I studied two different animal models : 1) The model of inactivation of the gene coding for Rspo1 and 2) The model of over expression of Rspo1 in the ovary. It was previously shown in my laboratory that Rspo1 is implicated in the development of the ovary. My project allowed us to show that defects in folliculogenesis following ovarian development, and more precisely abnormal follicles, were present in both models studied. In the model of invalidation the number of follicles is low and they are less organized and in the model of over expression the ovaries contain a high number of abnormal follicles, as well as cysts and tumoral masses in older individuals. In conclusion, my results suggest that Rspo1 is implicated in the folliculogenesis, which deffects can lead to different ovarian pathologies such as premature ovarian failure, cyst formation, and in some cases tumor formation. All these results allow us to better understand the physiology of the ovary and its pathologies.

Oral presentation

MiR-199a-5p, novel effector of the TGFbeta pathway.

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Keywords:

Fibrosis is the final common pathway in virtually all forms of chronic organ failure, including lung, liver, and kidney, and is a leading cause of morbidity and mortality worldwide. Fibrosis results from the excessive activity of fibroblasts, in particular a differentiated form known as myofibroblast that is responsible for the excessive and persistent accumulation of scar tissue and ultimately organ failure. Idiopathic Lung Fibrosis (IPF) is a chronic and often rapidly fatal pulmonary disorder of unknown origin characterized by fibrosis of the supporting framework (interstitium) of the lungs. Given the poor prognosis of IPF patients, new insights into the biology of (myo)fibroblasts is of major interest to develop new therapeutics aimed at reducing (myo)fibroblast activity to slow or even reverse disease progression, thereby preserving organ function and prolonging life. MicroRNAs (miRNAs), a class of non-coding RNA recently identified, are associated with normal cellular processes; and deregulation of miRNAs plays a causative role in a vast array of complex diseases. In this study, we identified a particular miRNA: miR-199a-5p that governs lung fibroblast activation and ultimately lung fibrosis. Overall we showed that miR-199a-5p is a major regulator of fibrosis with strong therapeutic potency to treat fibroproliferative diseases such as IPF.

Oral presentation

The role of Otx2 in the correct positioning of the midbrain-hindbrain barrier.

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Keywords: otx2, midbrain-hindbrain barrier, fgf8, adhesion molecules, isthmic organizer, gbx2

During embryonic development, the neural plate is regionalized anteroposteriorly into several domains with their own individual identities and fates. A correct positioning of the boundaries between those domains is crucial for a normal development of the nervous system. In order to study the mechanisms underlying this boundary positioning, we focused on the midbrain-hindbrain barrier (MHB), an already well-studied structure which involves a small number of well-known genes, including the Otx2 and Gbx2 homeogenes that are expressed early during embryonic development. The signaling molecules Wnt1 and Fgf8 are expressed later on at the MHB, respectively downstream of Otx2 and Gbx2, thus conferring to the MHB a role as a self-regulating organizing center for the surrounding domains, known as the isthmic organizer. Mutual repression between the anteriorly expressed Otx2 and the posteriory expressed Gbx2 was thought to be the earlier phenomenon determining the position of the MHB, and therefore the regionalization of both midbrain and hindbrain. Indeed, Wnt1 and Fgf8 expressions are initiated at the boundary between the areas of expression of Otx2 and Gbx2, and those former expressions in turn dictate the future position of the isthmic organizer. Using a conditional knock-in strategy for Otx2, we were able to shed a light on the shortcomings of this model : the position of the MHB and the correct development of the midbrain and hindbrain do not only depend on a mutual repression between Otx2 and Gbx2. When we triggered an ubiquitous expression of Otx2 in mutant mice, we observed that, contrary to what the model forecasted, the MHB was set in place in an anterior position, and that it lacked in sharpness. We are now trying to understand what functions those homeogenes could control that could further explain their role in the regionalization of the midbrain and hindbrain. One possibility is that Otx2 and Gbx2 could drive the expression of different adhesion molecules, thus giving rise to two cell populations with different adhesive properties. This could lead to the segregation of the two cell populations according to the homeogene they are expressing, and therefore to the creation of two separate domains.

Oral presentation

The polycomb protein BMI1 promotes cell proliferation through repression of cyclin protein expression in Chronic Myeloid Leukaemia Cells.

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Keywords: chronic myeloid leukaemia, bmi1, polycomb, cyclin protein

BACKGROUND: BMI1 is a polycomb protein involved in the epigenetic repressive control of essential cellular functions such as proliferation, senescence, metabolism and self-renewal in both hematopoietic and cancer stem cell. Interestingly, the expression level of Bmi1 in chronic myeloid leukaemia (CML) is directly correlated with disease progression. In our study we investigate the role of BMI1 in this disease and search news potential BMI1's targets. OBSERVATIONS: We developed an inducible shRNA system to silence Bmi1 in the human K562 CML cell line. In our cells, the Bmi1 down-regulation resulted in a reversible decrease in metabolic activity, proliferation and clonogenic potential, without induction of apoptosis. Furthermore, BMI1 is necessary for K562 tumor growth in a mouse xenograft model. A transcriptomic approach, between K562 cells with BMI1 downregulation or not, identified a gene coding for a cyclin protein as a potential target of BMI1. Interestingly, an inverse correlation between Bmi1 and this cyclin was measured in samples from patients both in the chronic and the acute phase of CML. Importantly, siRNA downregulation of this cyclin totally rescued the proliferation arrest and the clonogenicity defect but only partially the decreased metabolic activity induced by Bmi1 silencing. Consistently with these results, the overexpression of this cyclin resulted in a decrease of cell proliferation and clonogenic potential of CML cell lines. CONCLUSIONS: BMI1 contributes to cell proliferation, tumor growth and clonogenic potential of the leukemic cells. A cyclin appears as a new and important mediator of the action of BMI1 on cell proliferation and as a potential target of this protein in the CML disease progression.

Oral presentation

Cancer metabolism: exploiting disruption of AMPK and glycolysis as a potential therapeutic approach.

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Keywords: cancer metabolism, glycolysis, ampk, mcts, hypoxia, atp, oxidative phosphorylation

Many oncogenic signaling pathways contribute to alterations in cellular metabolism that fulfill the increased energy requirements of the growing tumour. The most obvious metabolic adaptation observed in most tumour cells is a shift in energy production from oxidative phosphorylation to aerobic glycolysis, described as the “Warburg effect”. Oxygen limitations in distant regions of the tumour from the blood vessels stabilize the hypoxia inducible factor 1 (HIF-1) which exacerbates the glycolytic pathway at the expense of mitochondrial respiration. This transcription factor increases glucose uptake, induces most of the enzymes of the glycolytic pathway, and the extrusion of lactate, the final product of glycolysis. Our strategy is to exploit the tumour cell glucose addiction for energy production through an inhibition of the export of lactate to decrease glycolytic ATP production. Lactate extrusion from the cell occurs via two members of the monocarboxylate transporter (MCT) family. MCT1 is constitutively expressed and MCT4 is strongly induced in hypoxia. We have already shown that an inhibition of these two transporters inhibits tumor growth but is not sufficient to kill tumour cells. Thus, in response to a reduced rate of ATP production through glycolysis, cells inhibit high-energy consuming processes (proliferation, protein synthesis,…) to maintain a stable level of ATP. We hypothesize that AMPK, which is a direct ATP sensor and “the guardian of energy balance” could be responsible for the cytostatic effect observed in response to MCTs inhibition. We expect that an inhibition of AMPK and MCTs could prevent tumour cells from sensing the drop of ATP and adapting to this metabolic disorder, leading to their death. We transformed an AMPK null mouse embryonic fibroblast cell line with H-RAS and showed that MCTs inhibition caused an accumulation of intracellular lactate and a dramatic inhibition of the glycolytic rate. Consequently, MCTs inhibition rapidly decreased the intracellular pool of ATP, more drastically in hypoxia than in normoxia, and lead to an inhibition of proliferation. However it was not sufficient to induce cell death in vitro. Finally, we showed that the inhibition of MCTs combined with phenformin, an inhibitor of oxidative phosphorylation, drastically reduced ATP levels and severely reduced cell survival. These findings suggest that a drastic inhibition of glycolysis, even in hypoxia is not lethal for the cells despite a major drop in ATP level even when the ATP sensor is inactive. In contrast the inhibition of MCTs with phenformin affects severely tumour cell indicating that tumour cells possess extensive metabolic redundancies to ensure their survival.

Oral presentation

Towards the deciphering of the genetic factors involved in durability of plant major resistance genes to root knot nematodes in pepper.

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Keywords: meloidogyne spp., capsicum annuum (pepper), me resistance genes, dosage allele effect, resistance durability, quantitative resistance

Root-knot nematodes (RKNs), Meloidogyne spp., are extremely polyphagous plant parasites worldwide. Since the use of most chemical nematicides is being prohibited, genetic resistance is an efficient alternative way to protect crops against these pests. However, nematode populations proved able to breakdown plant resistance and genetic resources in terms of resistance genes (R-genes) are limited. Sustainable management of these valuable resources is thus a key point of R-gene durability. In pepper (Capsicum annuum), Me3 is a dominant major R-gene, currently used in breeding programs, that controls M. arenaria, M. incognita and M. javanica, the three main RKN species. It was introgressed in either a susceptible or a partially resistant (i.e., that shows reduced symptoms) genetic background in either homozygous or heterozygous allelic status. Doux Long des Landes (DLL) was used as susceptible recipient pepper line and Yolo Wonder (YW) as a partially resistant one. Challenging all these genotypes with a high inoculation pressure of an avirulent M. incognita isolate demonstrated that i) the efficiency of the R-gene in reducing the reproductive potential of RKNs is strongly affected by the plant genetic background, ii) the allelic status of the R-genes has no effect on nematode reproduction. These results highlight the primary importance of the choice of both the R-gene and the genetic background into which it is introgressed during the selection of new elite cultivars by plant breeders.

Oral presentation

Use of hepatocyte death markers for the prediction of non alcoholic steatohepatitis in morbidly obese patients.

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Keywords: non alcoholic fatty liver, biological markers

Non Alcoholic Fatty Liver Disease (NAFLD) is the leading cause of liver disease in France. It is recognized as the representing hepatic manifestation of metabolic syndrome. The prevalence of NAFLD has risen rapidly in parallel with the dramatic rise in obesity and diabetes. NAFLD represents a spectrum of diseases ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), combining inflammation and more severe hepatocyte involvement, namely apoptosis and necrosis. These lesions may be associated with varying stages of fibrosis, which may ultimately progress to cirrhosis and hepatocellular carcinoma. While steatosis is considered benign and protective because it limits lipotoxicity, non-alcoholic steatohepatitis is, however, an advanced liver disease. Its early diagnosis is, therefore, an important issue in the management of NAFLD. The gold standard for diagnosing NASH is liver biopsy. A new non-invasive marker of hepatocyte apoptosis (Caspases-generated CK18 fragment) has been proposed as a predictor of NASH. This work aims to determine the value of the hepatocyte apoptosis marker (K18 fragment) as well as hepatocyte death (K18 total) and hepatocyte necrosis (total K18-K18 fragment) in the diagnosis of NASH, in morbidly obese patients. The serum levels of these markers (K18 total and K18 fragment) were evaluated in 362 morbidly obese patients. We have shown that the diagnostic value of these markers alone is insufficient. In order to find an explaination for this data, the role, of steatosis was also evaluated.. A direct correlation between the value of these markers and the degree of steatosis, with or without NASH, was identified. It was also noted that an overlap between major steatosis without NASH and low intensity steatosis with NASH may exist. This explains the difficulty in discriminating NASH from simple steatosis. The diagnosis of NASH may be facilitated by the association of the hepatocyte death marker with other biological parameters. The use of these markers alone does not allow for the diagnosis of NASH, but their potential integration into a score system would improve prediction and reduce the indication of biopsy for this pathology. In addition, determining the mechanisms by which steatosis, regardless of its degree, no longer plays its hepato-protective role would aid in identifying new players / markers of NASH.

Oral presentation

Implication of microRNA-34a in adipocyte insulin resistance during obesity.

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Keywords: mir-34a, adipocyte, p53, sirt1, vamp2, ptp1b, insulin, obesity

The adipose tissue (AT) dysfunction during obesity plays an important role in the development of insulin resistance. Recently, the implication of the anti-oncogene p53 has been demonstrated in functional alteration of AT, but the mechanisms are still unknown. In several cells, the microRNA miR-34a is important for p53 induced cell response. MiR-34a is known to target Vamp2 and Sirt1, two proteins implicated respectively in the translocation of glucose transporter (Glut) and insulin sensitivity. So we have investigated if some deregulation of miR-34a in obese AT could participate to insulin resistance. By using miRNA Q-PCR analysis we demonstrated that MiR-34a expression is increased in AT and isolated adipocytes from mice genetically obese or under high fat diet whereas Vamp2 and Sirt1 expression are decreased. To reveal the mechanisms, we developed an in vitro approach in 3T3-L1 adipocyte transfected with miR-34a or a miR control. Interestingly, miR-34a overexpression inhibits both Vamp2 and sirt1 expression and leads to a decrease in glucose uptake during insulin stimulation without change in Glut expression. Moreover, the activation of the insulin receptor, ERK and AKT was also found inhibited by this overexpression. Importantly, this effect is dependent of the tyrosine phosphatase PTP1B, protein known to be reduced by sirt1. Indeed, we found that miR-34a overexpression increase PTP1B expression leading to insulin signaling alteration that could be reversed by using anti-PTP1B siRNA. The miR-34a expression increase in adipocyte may be a new mechanism linking p53 activation and development of insulin resistance during obesity.

Posters

poster N°1

Role of the JNK pathway in epithelial morphogenesis.

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Keywords: drosophila dorsal closure, epithelial morphogenesis, , jnk signaling pathway, reprogramming

Drosophila dorsal closure (DC) is a two hour process, occurring at the end of embryogenesis, consisting on the movement of the two lateral ectodermal sheets over the dorsal tissue amnioserosa (AS) and it ends with a perfect fusion of them at the dorsal midline. The JNK signaling pathway, activated in a cell row at the leading edge (LE) of the ectoderm, is essential to this process. Fourteen segments compose the ectoderm, each one being divided in an anterior compartment and a posterior one that respectively expresses the patched and engrailed (en) genes. At the boundary between each segment, the groove cells are anterior cells (no en expression) and highly express the adherent junction protein enabled. The laboratory identified a singular type of anterior cells, called mixer cells (MC), that are localized at the intersection between the LE and the groove, being therefore JNK-positive and en-negative cells. During DC, these cells cross the boundary between segments, integrate in the adjacent posterior compartment, and simultaneously one to three cells from the row below intercalate in the LE. As a consequence tension occurring at the LE is released and DC can continue properly. The MC, just before crossing the segment boundary, is reprogrammed to express the posterior determinant en in a JNK-dependent manner. Inactivating JNK, in anterior cells, some segments do not fuse with the right counterpart causing segment misalignement. Interestingly the MC is not integrated in the adjacent posterior compartment. We are following two approaches to study the role of MC: one is a specific study on polycomb group genes (PcG) and the other is a more general RNAi approach. PcG are silencing genes acting on chromatin structure. JNK is known to downregulate the expression of these genes to induce change of cellular fate. I want to test therefore whether the MC reprogramming depends on PcG. In mutants for one PcG gene, polycomb (pc), MC do not cross the boundaries and some segments are misaligned, indicating a role of pc in the mixing process. In order to find new genes acting on MC reprogramming, I am performing a targeted RNAi screen. A selection of thirty JNK target genes sorted from a Microarray analysis was tested for this purpose. dsRNA were expressed in the anterior compartments of the embryo to affect the MC. RNAi for the gene spec2 gives a phenotype in DC in terms of segment mismatching. Preliminary results on a mutant for this gene indicate alteration of enabled expression mostly in the AS. However, mixing seems to occur properly. In conclusion results obtained on pc invoke a role of this gene in MC reprogramming. The de novo expression of engrailed in the MC could be a consequence of the chromatin remodeling that makes the gene accessible to transcriptional factors. On the other hand, spec2 is a promising candidate for further studies to elucidate its action on enabled localization, a protein known for its role on cytoskeleton organization. The two phases of the project will give new insight on epithelial morphogenesis.

poster N°2

Mechanisms of deficient oral tolerance in early life.

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Keywords: neonates, breastfeeding, vitamina, allergy

It is estimated that 300 million people have asthma, among which 2/3 are children and that an additional 100 million are likely to develop asthma by 2025. Chronicity of the disease and lack of curative treatment makes primary prevention a public health priority. We demonstrated in a mouse model that breastfeeding could prevent asthma by inducing oral tolerance in the breastfed progeny. Breastfeeding-induced tolerance required the presence of both allergen and tolerogenic co-factors, i.e. TGF-beta and/or allergen specific IgG in maternal milk. Recently, we found that full protection was observed only when antigen transfer occurs during the third week of lactation. Here we set out to investigate the mechanism responsible for defective oral tolerance induction during early days of life. We found decreased expression of retinaldehyde deshydrogenase (RALDH) in mesenteric lymph node CD103+ dendritic cells (DC) from 1 and 2 weeks old pups as compared to 3 weeks old. Supplementation of maternal diet with vitamin A during lactation increased RALDH levels in MLN CD103+ DC of pups aged of 1 and 2 weeks up to week 3 levels. In vitro ability of MLN cells to drive T cell proliferation and Th1 differentiation was also increased upon Vit A maternal supplementation. In parallel, we found in vivo, that maternal diet supplementation in Vitamin A during lactation decreased Th2 immune response in the progeny while regulatory T cells were not induced. On the contrary, we found increased Th1 immune responses in mice breastfed by mothers under Vitamin A enriched diet. These data indicate that, in contrast to what is described in the adult, RALDH increased expression in the neonate is associated with potentiation of Th1 (anti-infectious) but not regulatory immune responses.

poster N°3

Role of the TREK2 background potassium channels in nociception.

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Keywords: trek, nociception, thermosensitivity, nerve-skin, calcium imaging

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 TREK2 controls the perception of warm and cool-temperatures in mice and that each member of the TREK channels subfamily contributes to thermal perception in different temperature ranges. Our results reveal that together TREK1, TREK2 and TRAAK channels have complimentary roles in thermal- and mechanical-perception over a broad range of painful-conditions.

poster N°4

A GENOME-WIDE RNAI SCREEN TO IDENTIFY NEW REGULATORS OF HEDGEHOG SECRETION.

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Keywords: hedgehog, secretion, rnai screen, rab8

Hedgehog is a highly conserved and secreted morphogen which induces different cell fates at short and long ranges. It is known that hedgehog is dually lipidated, bearing a cholesterol moiety at its C-terminal and palmitic acid at N-terminal. But is not well understood how this highly hydrophobic protein can spread over many cells. Currently in the literature there are several hypotheses proposed for different trafficking pathways involved in the secretion of Hedgehog. Hedgehog is proposed to be transported on lipoproteins, also by forming multimers, transport on exosomes etc. The transporter, dispatched, is the only known protein to be dedicated to hedgehog secretion. To identify novel proteins regulating the secretion of Hedgehog, we developed a genome-wide RNAi screen using the Drosophila wing imaginal disc as a model. We screened the VDRC KK RNAi transgenic line collection which target most of (80%) the Drosophila genome. We had a testing environment in which Hedgehog was overexpressed in the producing cells. Hedgehog overexpression per se leads to pupal lethality, but this phenomenon can be rescued by introduction of Dispatched RNAi. Hence, we screened for the suppressors of lethality and obtained 76 (0.707%) candidates out of the 10,700 lines screened. In this group, we have genes which regulate the cell growth and division, genes involved in exocytosis, and a few of unknown function. On an average, we have about 20% of lines showing an enhancement of lethality. I will present our RNAi screening strategy and one of our strong positive hits – Rab8 GTPase. Rab8 is a monomeric GTPase regulating the delivery of proteins to apical / basolateral surface. Rab8 RNAi and mutant rescue the lethality caused by overexpression of Hh. Rab8 downregulation also affects the localization of endogenous Hh. I am currently characterizing and analyzing Rab8 further by looking at its role in regulation on the subcellular Hedgehog localization and release.

poster N°5

Anticancer immune response participate to HIPEC (Hyperthermic IntraPeritoneal Chemotherapy)-induced protection of patients suffering from peritoneal carcinomatosis.

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Keywords: cancer, immunity, hsp

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 aims of our work are to uncover how HIPEC could enhance the patient's survival? Using tumor cells of patients suffering from peritoneal carcinomatosis of colorectal origin obtained before and after HIPEC, we observed an overexpression of Hsps in response to treatment. Interestingly, we found that this expression is specific to the tumor tissue, as it was not observed in the healthy tissues obtained from the same patient. We also observed a re-localisation of Hsp70 from the cytosol to the plasma membrane of the tumor cells. We focused on Hsp70 as it is known it plays a key role in the induction of anticancer immune responses, and we speculated that the immune system could participate in the protective effect brought by HIPEC. Therefore using murin colon carcinoma cell line (CT26) we showed that HIPEC could increase Hsp70 expression in tumor cells. Interestingly there is no difference between HIPEC and chemotherapy alone even in the activation of T cells. When mice were immunized with chemotherapy-treated dead tumor cells, they could be best protected from a subsequent challenge using the same tumor in viable form compared to mice immunized with HIPEC-treated dead tumor cells (vaccination assay). Taken together, our results demonstrate that 1/ HIPEC leads to an expression of Hsps to the plasma membrane of tumor cells and 2/ that HIPEC-treated cells can vaccinate some mice against tumor development but less efficiently that high chemotherapy. We are currently investigating the role of Hsp70 in this protection.

poster N°6

Molecular mechanisms of brown and white adipocyte generation during differentiation of human induced pluripotent stem cells.

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Keywords: adipocyte progenitor, hips, pax3, retinoic acid, development

Brown and white adipocytes are both involved in the energy balance but assume opposite functions. The discovery of functional brown adipocytes in adult humans offers promising prospects for clinical medicine, since recruitment of brown adipocytes could constitute a valuable therapeutic approach to counteract obesity. However, regulators of white and brown adipocyte progenitor (AP) abundance during development remain to be characterized in humans. In this study, we used the adipogenic potential of human induced Pluripotent Stem (hiPS) cells to identify pathways and molecular mechanisms that could regulate generation of brown and white adipocytes. We established conditions to induce hiPS cell adipocyte differentiation. Then, we derived brown and white APs and characterized them at the molecular level. We performed retroviral infection assays to identify a molecular mechanism involved in AP specification. Retinoic acid (RA) pathway activation at an early phase of hiPS cell differentiation promoted white adipocyte generation and inhibited brown adipocyte lineage. In contrast, TGF? pathway repressed the white adipocyte phenotype and was required for brown adipocyte generation. The selective generation of brown and white adipocytes allowed us to set up a procedure to derive both APs. Pax3 transcription factor was enriched in brown APs and Pax3 overexpression in white APs induced the brown adipogenic gene programme. Together, these data support a model in which RA and TGF? pathways regulate the generation of white and brown APs in an opposite manner. Pax3 plays a critical role in human AP specification and brown adipocyte differentiation.

poster N°7

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

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Keywords: cancer, stem cells, hiv-protease inhibitors, oct4, apoptosis, stemness signature, lopinavir

Objectives: 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 (1). 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 pportunities for patients suffering from solid cancer tumours of poor prognosis. References : (1) Darini, CY, Pisani, DF, Hofman, P, Pedeutour, F, Sudaka, I, Chomienne, C, Dani, C and Ladoux A., (2012) Self-renewal gene tracking to identify tumour-initiating cells associated with metastatic potential. Oncogene 31: 2438-49.

poster N°8

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

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Keywords: anoxia, eif5a, gc7, mitochondria, mitochondrial silencing

The eukaryotic initiation factor 5A (eIF5A) is highly conserved throughout evolution and its unique characteristic is its post-translational activation through hypusination. This modification is sequentially catalysed by two enzymatic steps involving 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. We also studied the effect of GC7 on mitochondrial status and shown that GC7 induced a reversible “mitochondrial silencing” characterized by a decrease in mitochondrial potential (??m) 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.

poster N°10

Evaluation of the sterol/PI(4)P exchange activity of Osh proteins.

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Keywords: osh proteins, yeast, sterol, pi(4)p

Osh proteins in yeast (ORPs in human) integrate a sterol-binding OSBP-related domain (ORD) and were thought to be mere sterol transporters or to act as sensors of cellular sterol levels. However we found recently [1] that the archetypal Osh/ORP, Osh4p, exchanges sterol for PI(4)P between lipid membranes. We propose a new model in which Osh4p transports these two lipids along opposite routes between the ER and the trans-Golgi. Interestingly, sequence analysis and homology models suggest that the ability to extract PI(4)P is likely highly conserved among Osh/ORP proteins. To validate this hypothesis we are now testing other proteins of the Osh family for their biochemical and structural properties. We expressed and purified full-length Osh5p, Osh6p and Osh7p and the Osh3p ORD and tested whether they were able to extract in vitro DHE and PI(4)P and to exchange these lipids between two liposome populations. To monitor this, we notably created a novel protocol to follow the transport of these two lipids in real time by fluorescence. Our results showed that Osh5p exchanges sterol for PI(4)P between lipid bilayers as Osh4p does, but, surprisingly, that the Osh3p ORD, Osh6p and Osh7p only transport PI(4)P. We aim now to better explain these functional differences at the structural level. This work constitutes a step towards redefining the primary role of Osh proteins/ORP. One particularly appealing hypothesis is that Osh/ORP proteins constitute a family whose common function is to use PI(4)P to transport/regulate other lipids in eukaryotes. [1] de Saint-Jean M et al. J Cell Biol. (2011) 195 : 965-78.

poster N°11

Role of metformin on lipid metabolism in prostate cancer cells.

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Keywords: prostate cancer, metformin, lipogenesis, metabolism

Cancer and type II diabetes are two diseases that appear to be associated. Retrospective epidemiological studies and preclinical data show that metformin, a drug commonly used in type II diabetes, displays antitumor properties. Deregulation of lipid metabolism is a well recognized hallmark of cancer cells and elevated levels of lipogenic enzymes as well as increased lipogenesis has been reported in a wide variety of cancers, including prostate cancer. In this report, we investigated the effect of metformin on lipogenesis in prostate cancer. Using different in vitro approaches, we found that metformin inhibits lipogenesis in several prostate cancer cells. This inhibition is correlated with a decrease of expression of mRNA and proteins involved in lipogenesis such as SREBP1c (Sterol Regulatory Element-Binding Protein 1c) and FAS (Fatty Acid Synthase) and with the inhibition of ACC (Acetyl CoA carboxylase) activation. Further, we demonstrate that this process is dependent on the activation of AMPK (AMP- activated protein kinase) a major regulator of energy metabolism. Importantly, we showed that overexpression of SREBP1c which controls lipogenesis, doesn't reverse the inhibitory effects of metformin on lipogenesis. Indeed, we demonstrate that metformin inhibits the synthesis of malonyl CoA the precursor of fatty acid. In conclusion, our results describe a new mechanism of action of metformin in prostate cancer cells.

poster N°12

Genetic correction of Xeroderma Pigmentosum skin stem cells.

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Keywords: xeroderma pigmentosum, genetic disease, cancer, skin, uv, dna repair, gene therapy

Xeroderma Pigmentosum (XP) is a rare monogenic genodermatosis (autosomal, recessive) characterized by an extreme photosensitivity associated with the development of numerous malignant epidermal carcinoma and melanoma in photo-exposed skin. XP cells are deficient in Nucleotide Excision Repair (NER) of DNA mutagenic lesions induced by ultraviolet (UV) exposure. The disease is heterogeneous and falls into 7 groups of genetic complementation (XP-A to XP-G). The XP-C group accounts for about 50% of XP patients. It is the most represented in Europe and the clinical traits are limited to skin cancer proneness without neurological problems observed in other groups. XP patients frequently receive plastic reconstructive surgery by autologous grafting. However these grafted epithelia remain repair deficient and thus cancer prone. In absence of efficient pharmaceutical treatment for these patients, our project is to genetically correct patients' cells ex vivo before grafting epithelia sheets composed only of corrected cells. We developed a procedure to express the wild-type XPC gene together with the CD24 gene using retroviral infection. CD24 is a small cell surface protein naturally expressed in post-mitotic epidermal keratinocytes (Magnaldo and Sarasin, 2001). Corrected keratinocytes are selected with a specific anti-CD24 antibody. This allowed us to obtain a population of cells corrected at homogeneity and enriched in stem cells (Bergoglio et al, 2007; Warrick et al, 2012). The expression of the therapeutic gene was maintained after serial propagation (>150 Population Doubling - PD). We have demonstrated that these corrected cells express the XPC protein, they recovered full DNA repair capacity and UV cell survival. These characteristics were retained in organotypic skin cultures as well as in graft onto athymic mice. This is the first preclinical model of ex vivo cutaneous gene therapy for XP-C patients. These very encouraging results led us to improve transfer vector, in order to fulfill specifications for grafting XP-C patients. As previously, corrected cells will be analyzed for appropriate gene expression, DNA repair and sustained capacity to develop normal skin fully protected against UV induced cancers after chronic or acute irradiations. Profile of genome expression in corrected cells will be assessed by pangenomic transciptome analyses. Whether reexpression of XPC, in XP-C patients' cells, triggers an immune response will be determined by ELISPOT. Our research opens the first realist perspective of corrective gene transfer ex vivo in skin of XP-C patients.

poster N°13

DEVELOPMENT OF AN EXPERIMENTAL BONE METASTASES MODEL.

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Keywords: bone, metastases, biomaterial, rat model, surgical method

Most cancers have the ability to metastasize to the bone, particularly breast tumors. In most cases, these metastases are linked to a massive bone resorption leading to numerous pathological events such as bone pain or risks of fracture, which often require resection surgeries with bone defect filling. Despite the therapeutics progresses accomplished lately, bone metastases are still associated with a poor prognosis for the patient. The aim of our study is to test the therapeutic potential of a bone defect filler biomaterial paired with different molecules of interest, mainly a biphosphonate (BP) or a chemokine (Graftys Laboratory collaboration). BPs have shown their utility in bone cancer treatment due to their ability to decrease the number of pathological events, and the decision to pair a chemokine with the calcium phosphate cement came from the intention to stimulate the patient's immune system. Hence the necessity to develop a model of breast cancer's bone metastasis, based on a rat model of bone implant. The choice of the rat as an animal model is dictated by the need to implant the biomaterial in bones of sufficient size. Cells of rat mammary carcinoma (MRMT-1 cells line) are implanted in the femur of Sprague-Dawley females, creating a syngeneic system. The osteolytic lesion development is monitored by radiography, allowing us to determine the stage at which the partial tumor resection must be done. The bone defect thus created is then filled with a biomaterial containing the molecule of interest, and the resumption of tumoral activity is monitored by radiography. This approach of localized delivery of molecules of interest on the lesion sites should allow us to decrease, or even to prevent altogether, the secondary effects linked to the usual treatments systemic delivery. Moreover, the simultaneous use of cements paired with different molecules (BPs, chemokines) could help to potentiate the effects of each of these molecules.

poster N°14

Delta-Notch signaling regulates apico-basal polarity in the zebrafish neural tube.

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Keywords: apico-basal polarity, notch signaling, mindbomb, endocytosis, zebrafish

Delta-Notch signaling is of primordial importance for embryonic development and adult tissue homeostasis. While it is well established that endocytic transport of Delta ligands is essential for Notch signaling, the reason for this requirement remains mysterious. Recent work suggests that endocytosis is important in polarized epithelial cells to ensure basal-to-apical transport of Delta ligand molecules that would be important to allow their productive interaction with apically localized Notch receptors. We have established a novel live imaging assay that reveals a highly dynamic movement of Delta-containing endosomes along the apico-basal axis of the neuro-epithelium, in accordance with a potential importance of apico-basal transport for vertebrate neurogenesis. To investigate the role of endocytosis in DeltaD trafficking, we inhibited function of the ubiquitin ligase Mindbomb, which is essential for endocytosis of DeltaD ligands and activation of Notch signaling. To our surprise, we discovered that loss of Mindbomb led not only to a lack of endocytic DeltaD internalization, but also to a loss of apico-basal polarity in the spinal cord. We will present evidence that this phenotype is indicative of an unexpected novel role of canonical Notch signaling in the establishment of apico basal polarity in the zebrafish neural tube.

poster N°15

Beta-cell regeneration induction using a chemical compound.

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Keywords: beta cell, regeneration, diabetes, mouse, endocrine pancreas

Background and aims: Type 1 diabetes arises as a result of a cell-mediated autoimmune destruction of insulin-producing pancreatic ?-cells. One avenue of research for a potential therapeutic strategy is cell replacement therapy using cell differentiation/reprogramming to turn different cells sources into ?-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 ?-like cells through the constitutive/ectopic expression of a single gene, Pax4 (a gene involved in the embryonic specification toward the ?-cell fate). More recently, we demonstrated that the misexpression of Pax4 in glucagon-expressing cells age-independently induces their conversion into ?-like cells. The regenerative capacity of glucagon-producing cells and their potential for conversion into ?-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 ?-cells into ?-like cells in vitro. In vivo tests were then initiated using Glu-Cre::Rosa26-lox-?-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, ?-like cells are regenerated following streptozotocin treatment, these deriving from cells that expressed the glucagon hormone.

poster N°16

First insights into the genetic diversity of the pinewood nematode in its native area and around the world.

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Keywords: bursaphelenchus xylophilus, microsatellite, genetic structuration, invasion routes

Biological invasions provide a great opportunity for research in evolutionary biology and specifically in short-term evolutionary process. For example, understanding the factors of success or failure of introductions and establishments in remote areas can improve our knowledge on both adaptation dynamics and colonisation processes in the context of invasions. Identifying the invasion routes and determining the origin of new outbreaks are of crucial importance and are a pre-requisite to address these questions. This may also permit to improve or establish regulatory measures and to potentially limit the damage. The pinewood nematode (PWN), Bursaphelenchus xylophilus is the causal agent of the pine wilt disease and is currently considered as one of the most important pests and pathogens in forest ecosystems. Native to North America, it has been introduced and it has spread in pine forests in Asia and Europe where it has now considerable economic and environmental impacts. In order to decipher the invasion routes and to improve our knowledge about this specific case of invasion using population genetic approaches, we have developed a set of PWN-specific microsatellite markers, usable in routine conditions at the individual level. Preliminary results on a set of samples from the native (North America) and invasive (Europe and Asia) areas indicate: (i) a fine spatial genetic structure at the scale of the pine tree and probably at larger scales in the native area and (ii) a very low level of polymorphism in PWN populations from invasive areas. The genotyping of samples from more representative areas in Europe, Asia and North America is currently underway in the laboratory in order to confirm/refine these conclusions. Assessing the genetic diversity of populations constitutes the cornerstone to determine the source of the European invasive PWN populations and whether they are the result of a single or several independent events of introduction.

poster N°17

TRF2 is overexpressed in human tumor vessels and contributes to endothelial cell angiogenic functions.

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Keywords: trf2, angiogenesis, telomere, wt1

We report here that TRF2 is overexpressed in endothelial cells of tumor vessels in different human cancer types, but not in cells of the vasculature 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. Since the transcription factor WT1 (Wilms' 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 in luciferase reporter assays. Additionnally, we found that TRF2 dosage modulates the expression of PDGFRbeta, a tyrosin kinase receptor, at the transcriptional level. We propose that TRF2 regulates the expression of PDGFRbeta, known to be important for proliferation, migration, and tube formation of endothelial cells.

poster N°18

The Anti-apoptotic Bcl-B Protein Drives Multiple Myeloma Pathogenesis Through Plasmocyte Proliferation and Differentiation.

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Keywords: multiple myeloma, bcl-b, plasma cells, bone marrow

Multiple Myeloma (MM) is a malignant condition that evolves from Monoclonal Gammapathy of Undetermined Significance (MGUS) and corresponds to the expansion of abnormal plasmocytes in the bone marrow. However, the factors underlying the malignant transformation of plasmocytes in MM are not fully characterized. We report here a MM phenotype in transgenic mice with Eβ-directed expression of the Bcl-B (BCL2L10) protein, an anti-apoptotic member of the Bcl-2 family. With age, Eβ-Bcl-B transgenic mice develop characteristic features of human MM, including elevated serum Ig, increase of plasma cells in the Bone Marrow (BM), anemia, characteristic bone lytic lesions and amyloid kidney deposits. Furthermore, transcriptional profiles of Eβ-Bcl-B bone marrow B cells show increased expression of known human MM dysregulated genes. Importantly, Bcl-B is overexpressed in purified bone marrow CD138+ cells from MM, but not MGUS or healthy patients, strongly suggesting that Bcl-B drives MM. The similarities of this model with the human disease, together with Bcl-B overexpression in human MM, identify this protein as an essential actor of MM pathogenesis.

poster N°19

Genetic disruption of CD147/ Basigin, a subunit of lactate-H+ symporters (MCTs) sensitizes glycolytic tumor cells to phenformin.

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Keywords: basigin, cancer, monocarboxylates transporters, lactic acid, glycolysis, atp, phenformin

In response to the hypoxic tumor microenvironment, tumor cells shift their metabolism towards glycolysis leading to a high production of lactic acid, which is efficiently exported by the MonoCarboxylate Transporters (MCTs). These MCTs (1 and 4) are H+/lactate symporters that require an interaction with an ancillary protein, CD147/Basigin, for their plasma membrane expression and function. Basigin is a conserved transmembrane glycoprotein that is strongly expressed in several tumor types. Considering the multiplicity of functions and interactions of CD147/Basigin, its role in promotion of tumour growth has remained poorly defined. To gain insight into this question, we designed experiments using Zinc Finger Nuclease (ZFN)-mediated basigin and mct4 knockouts in the colon adenocarcinoma (LS174T), the lung carcinoma (A549), and the gioblastoma (U87) human cell lines. First, we demonstrated that the major protumoral action of CD147/Basigin is to control the energetics of glycolytic tumours via MCT1/4 activity. Second, we showed that basigin gene knockout reduced the plasma membrane expression of MCT1/4 and lactate transport. As a consequence of this decrease, cells accumulated a large pool of intracellular lactate and redirected part of their energy metabolism towards oxidative phosphorylation. This glycolytic/MCT-block ‘escape’ allowed these tumor cells to display residual growth in vitro and in vivo. Thirdly, we found that in contrast to tumour parental cells, their derivatives basigin-/- or basigin-/- and mct4-/- became highly sensitive to phenformin, an inhibitor of mitochondrial complex I. Phenformin addition to these Basigin/MCT disrupted cells in normoxic and hypoxic conditions induced a major drop in cellular ATP provoking growth arrest and cell death via ‘metabolic catastrophy’. These findings highlight that a major protumoral action of CD147/Basigin is to control the energetics of glycolytic tumors via MCT1/MCT4 activity and that blocking lactic acid export could provide an efficient anticancer approach, in particular when combined with phenformin.

poster N°20

A vapBC-type toxin-antitoxin module of Sinorhizobium meliloti influences nitrogen fixation and senescence phenotype in symbiosis with an agronomic plant of interest Medicago sativa.

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Keywords: symbiosis, toxin antitoxin system, sinorhizobium meliloti, medicago sativa, senescence, nitrogen fixation

The nitrogen-fixing symbiosis between the legume, Medicago sativa and the bacterium, Sinorhizobium meliloti leads the development of a new root organ, the nodule. In this organ, the bacterium differenciated on fixing bacteroid supplying in the plant a source of nitrogen. In return, the plant supplies in the bacteroid the carbon substrata. However, this nodule presents a premature senescence with regard to other organs of the plant, which begins with the death of bacteroids, followed by that of the infected plant cells. This leads to the ruling of the nitrogen-fixing. To understand the role of the bacterial partner in this nodule senescence, we were interested in the systems "Toxin-antitoxin" (TA). To the animal pathogenic bacteria, certain TA systems are described as occurring in the survival of bacteria in the host cell as well as in the adaptation to different stress. A TA system consists of an unstable antitoxin and a stable toxin which, during a stress, acts as regulator of the translation. We examined the possible role of S. meliloti VapBC (Virulent Associated Protein) modules in bacteroid viability and nodule senescence. In this study, we examined the effect of a Tn5 insertion in the toxin component of a S. meliloti vapBC locus during bacterial interaction with Medicago sativa. We showed that, during the symbiotic interaction, the bacterial mutant affected in the toxin vapC5 have a nitrogen-fixing activity 30% more than in a wild type, with also an increase of plant yield and a delayed senescent phenotype. The role of "Toxin" in the symbiotic interaction will be discuss.

poster N°21

Cascading effects of nitrogen and water input on plant-herbivorous insect interactions.

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Keywords: plant-insect interactions, nutritional value, plant defense, nitrogen, water, plant vigor hypothesis, n limitation hypothesis, tuta absoluta, leaf n, leaf c/n

In general, plants face a dilemma when they experience various biotic attack (i.e. plant disease, birds, insects, etc.) or abiotic stress (i.e. nutrient, water and sanity etc.): to grow or defend (Herms and Mattson 1992)? Specifically, plant-herbivorous insect interactions have become a central research topic at this context. In our case study, the effects of different levels of nitrogen (N) and water input to the tomato plants (Solanum lycopersicum) on survival and development of a tomato leafminer, Tuta absoluta (Meytick) (Lepidoptera: Gelechiidae) were investigated under laboratory conditions. N limitation (or excessive), water stress and their interactions significantly reduced T. absoluta survival rate, pupal weight and caused delayed development. Plants treated with insufficient or excessive N demonstrated a sub-optimal growth (smaller in plant height and less numbers of nodes). In particular, in the case of optimal N vs. insufficient N, a lower level of leaf N content and higher level of ratio of carbon to nitrogen (C/N ratio) were identified in plants treated with insufficient N. We assumed that a combination of poor nutritional value and higher level of chemical defense may explain the observed adverse effect on T. absoluta. Therefore, our findings provided new evidences for both “Plant vigor hypothesis” (Price 1991) and “N limitation hypothesis” (White et al. 1993) that herbivorous insects perform better on rapidly growing (vigorous) plants and N input to plants could be a limiting nutrient factor to growth and development of Lepidoptera insects.

poster N°22

In vitro screening of thyroid function disruption: PCCl3 is a promising tool.

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Keywords: thyroid, endocrine disruption, screening, in vitro, iodine

The evaluation of the endocrine disrupting properties of chemicals has become a necessity for the chemical industry. The focus has until now mainly been put on sexual hormones disruption, and early in vitro screening tests are already available and validated to assess disruption at the Hypothalamic-Pituitary-gonadal axis. This is not the case for effects on thyroid function disruption. The aim of this work is to provide a test suitable for a rapid, cheap and “test product-saving” detection of thyroid hormone synthesis disruption. Thyroid hormone synthesis occurs in thyroid follicles, a three dimensional structure made of thyrocytes, and involves specific proteins. Toxicity against one of these proteins or disruption of hormone synthesis leads in most of the cases to the deregulation of the transcription of corresponding genes. A rat thyrocyte cell line, PC-Cl3, appears to be able to reproduce in vitro the profiles of deregulation of gene expression observed in vivo after administration of thyroid hormones synthesis disruptors. Here we tested the hypothesis that mRNAs of thyroid specific genes are deregulated in a similar manner in rat in vivo and in the studied cell line when exposed to iodine excess. We exposed male Wistar rats to 0.05% sodium iodide in drinking water (? 37.5 mg/kg/d) for 6 consecutive days. This exposure time and dose were sufficient to observe significant changes in blood thyroid hormone levels. Thyroids have been collected for qPCR investigations. Sodium-Iodine Symporter (NIS), Thyroperoxydase (Tpo) and thyroglobulin (Tg) thyroid specific genes-related mRNAs expressions were significantly down-regulated and pendrin mRNA expression was significantly up-regulated. The exposure of PC-Cl3 cells for 72h to 10-5M sodium iodide in culture medium leaded to a comparable deregulation profile. These results are indicating that this particular cell line may be relevant for the elaboration of an in vitro screening test for the thyroid hormones synthesis disruption.

poster N°23

KEY ROLE PLAYED BY SOX11 DURING NEPHROGENESIS.

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Keywords: kidney development, nephrogenesis, cakut, sox11

Kidney development results from a complex combination of morphogenetic processes and can be divided into two main parts: 1) Growth and branching of the ureter (forming the future collecting tubules) and 2) Nephrogenesis that gives rise to the renal functional units. The branching process starts with the outgrowth of the ureteric bud (UB) from the caudal part of the Wolffian duct (or nephric duct) into the metanephric mesenchyme (MM). Continuous signalling from the mesenchyme stimulates UB growth and induces a series of dichotomic branching events to give rise to the tree-like collecting duct system. Nephrons originate from the MM compartment with mesenchymal cells condensing around the UB tip to form a pre-tubular aggregate. Aggregates epithelialize through a mesenchymal to epithelial transition and quickly adopt a patterned organization. This patterning, that involves asymmetric cell divisions, leads to the evolution of renal vesicles into comma and S-shaped bodies in which precursors of nephrons segments (glomerulus, proximal tubules, Henles' loop and distal convoluted tubules) have already adopted a partially differentiated state. Disruption of any of these events can lead to Congenital Abnormalities of the Kidney and the Urinary Tract (CAKUT), which represents one of the most common birth defects in human and includes renal hypo-dysplasia, hydronephrosis, hydro-ureter, duplex kidneys, cysts etc. SOX genes (for SRY related high mobility group box) encode transcription factors that are essential for organogenesis, sex determination (SoxA), erythropoiesis (Sox6), skeletal, neural and mesenchyme development (Sox9) and also required for kidney development. Our laboratory has previously shown that Sox8/9 plays an important role in ureter branching. Sox11 is expressed in both the UB and MM progenitors, but at later stage becomes restricted to the intermediate segment of developing nephrons: this highly dynamic expression pattern may suggest specific roles in the different compartments. In order to study the function of Sox11 during kidney morphogenesis, we made use of a CRE/LoxP strategy to specifically delete Sox11. Our data demonstrate that Sox11 depletion leads to a range of kidney abnormalities, depending on the compartment affected: mutant embryos show an early malformation in the first steps of morphogenesis (ectopic UB emergence) resulting in bifid-duplex kidney, and a late phenotype affecting the MM derived structures (nephrons) resulting in a smaller kidney. My study focuses on the second aspect of SOX11 function. Our results show that Sox11-/- kidneys are characterized by shortened tubular segments (proximals tubules and Henle's loops) that eventually dilate in pre-cystic structures. I have also shown that these malformations are not due to aberrant branching, but that they are related to defective elongation of the tubular segments and probably linked to abnormal differentiation of nephron precursors. My future results will clarify the molecular mechanisms underlying the defective nephrogenesis in order to better elucidate the role of Sox11 during nephron differentiation and maturation.

poster N°24

HMGA2 deregulation in adipocytic tumors.

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Keywords: hmga2, aadipocytic tumors, lipomas, liposarcomas, cytogenetic, ppap2b

Adipocytic tumors (AT) are the most frequent human mesenchymal tumors. They include benign and malignant entities. Genetic studies are powerful for improving classification and clinical diagnosis of AT. HMGA2 (High Mobility Group A2) gene is localized on the long arm of the chromosome 12 (12q14.3) and encodes a non-histone chromatin protein. The first three exons code for structural DNA-binding domains. HMGA2 is expressed only during early development in humans. 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 benign lipomas. Recent results from our team have shown that exons 1-3 of HMGA2 were systematically amplified in a series of 38 malignant AT, suggesting a crucial role for HMGA2 in AT pathogenesis. 1) We studied HMGA2 regulation and role of let-7 miRNA in lipomas. HMGA2 mRNA was overexpressed in 100% and 76% of lipomas with and whithout structural rearrangement of HMGA2, respectively. These results showed existence of deregulation mechanisms other than HMGA2 structural rearrangements. We studied the expression of 8 members of let-7 family 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 (Bianchini, Saada, et al. Genes Chromosomes Cancer, 2011) . 2) We analyzed a case of lipomatosis, a rare proliferative disorder of the adipocytic tissue. We detected neither structural rearrangement of HMGA2 nor HMGA2 mRNA and protein overexpression. In contrast, we observed an inhibition of expression of let-7 miRNA, suggesting a role for let-7 in HMGA2 regulation in lipomatosis (Saada, et al., Histopathology, 2012) 3) We identified PPAP2B gene as a novel fusion gene partner of HMGA2 in lipomas using FISH-based positional cloning strategy and reverse transcription-PCR (RT-PCR) analyses in a series of lipomas showing chromosomal translocation t(1;12)(p32;q14). Because PPAP2B is member of the lipid phosphate phosphatases family, it may play a proper role in lipoma pathogenesis. Indeed only five fusion partners of HMGA2 in lipomas have been identified so far and it is not resolved whether the overexpression of HMGA2 in those lipomas may be due to the loss of negatively acting domains located in the 3'UTR of HMGA2 or to the specificity of chimeric sequences resulting from the fusion (Bianchini, Birtwisle, Saada et al., Genes Chromosomes Cancer, 2013). 4) We studied by FISH a series of 117 malignant atypical lipomatous tumors and dedifferentiated liposarcomas containing amplification of MDM2. We detected amplification of exons 1-3 of HMGA2 in 88% of tumors, which was statistically linked with good prognosis factors and adipocytic differentiation. CDK4 (12q14) and JUN (1p32) amplification were detected in 69% and 42% of tumors respectively. Their amplification was associated with dedifferentiated subtype and bad prognosis features. Our original results confirm the early role of HMGA2 in atypical lipomatous tumors and dedifferentiated liposarcomas genesis. JUN and CDK4 may act as secondary genetic alterations conferring proliferative advantages to liposarcoma cells (Saada et al., manuscript in preparation).

poster N°25

Antigen Specific ELISA to Improve Anti-PLA2R1 antibody Monitoring after Renal Transplantation for Membranous Nephropathy.

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Keywords: membranous nephropathy, anti-pla2r1 antibodies, renal transplantation, recurrence, elisa

Background: Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults and leads in 40-50% of cases to end-stage kidney disease (ESKD) in the long term. MN represents 2% of the patients on waiting list for kidney transplant. Recurrence of MN after renal transplantation has been reported to occur in 7-42% of patients in various studies, resulting in reduced allograft survival. MN is defined by subepithelial immune deposits containing IgG (mainly IgG4) and complement fractions with alteration of the basement membrane structure. In 2009, Beck et al. identified the M-type phospholipase A2 receptor (PLA2R1) as the first podocyte antigen involved in adult idiopathic MN. The presence of autoantibodies directed to PLA2R1 has been confirmed in 65 to 82% of patients with MN. The pathogenic role of anti-PLA2R1 antibodies is not yet proven, but their titers seem to correlate with MN activity in native kidney. The interest of anti-PLA2R1 to predict recurrence of MN in kidney recipiens remain controversial. Methods: We have validated in a cohort of 102 MN patients and 51 controls an antigen specific ELISA, and compared with Indirect ImmunoFluorescence (IIF) on HEK cells transfected with PLA2R1. ELISA had higher sensibility and accuracy (Se: 67% Spe: 100%) than IIF (Se: 58% Spe: 100%) and we confirmed that the titer of anti-PLA2R1 antibodies is correlated with the activity of the disease. Our aim was to monitor PLA2R1 antibodies using this ELISA on a retrospective case series of 13 kidney transplant recipients with MN who had serial sera available during follow-up, and to test the correlation between anti-PLA2R1 antibody titers and clinically significant MN recurrence. Results. Nine patients (69%) had anti-PLA2R1 antibodies at the time of renal transplantation. One patient had persistent anti-PLA2R1 activity after transplantation, relapsed, and was successfully treated with Rituximab. Another patient with persistently high PLA2R1 antibody titers had histological relapse but no proteinuria on treatment with renin angiotensin system inhibitors. These two patients who exhibited MN recurrence had high anti-PLA2R1 activity at the time of renal transplantation as well as a subsequent mild immunosuppressive regimen: i.e. either no induction therapy or no calcineurin inhibitors. The seven other patients exhibited a decrease of their PLA2R1 antibody titers following a strong immunosuppression for renal transplantation and did not relapse, including two who had an increase of proteinuria with another nephropathy. One out of four patients without PLA2R1 antibodies at the time of transplantation relapsed, suggesting the presence of autoantibodies directed against another antigen target. Conclusions. The presence of anti-PLA2R1 at the time of kidney transplantation does not imply recurrence after a strong immunosuppressive regimen. The monitoring of anti-PLA2R1 titers during follow-up helps to predict MN recurrence, as well as to diagnose other nephropathies.

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Involvement of FMRP sumoylation in spinogenesis.

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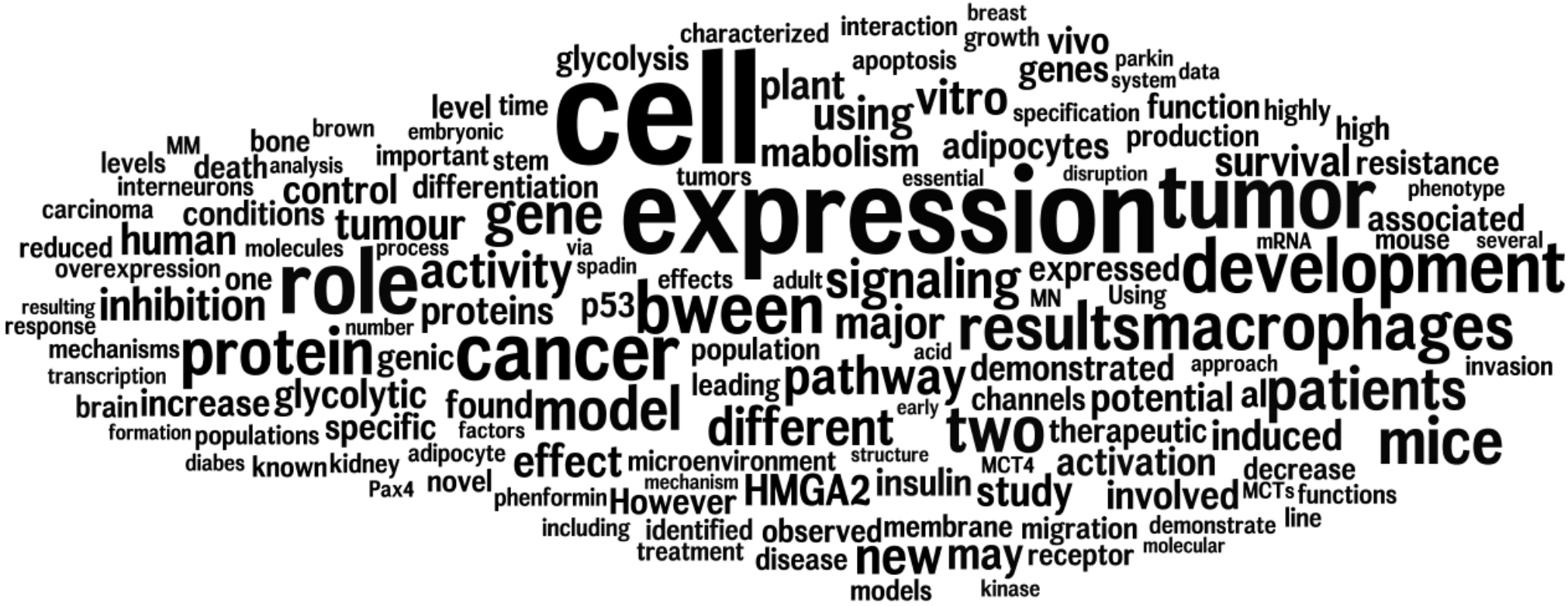
Fragile X Syndrome (FXS) is the most frequent inherited cause of intellectual disability in children and is caused by the lack of expression of the mRNA-binding Fragile-X Mental Retardation Protein (FMRP). FMRP plays a role in the activity-dependent targeting and translation of specific mRNAs in dendrites. The absence of FMRP expression in neurons leads to an abnormal neuronal morphology with increased spine length and density. FMRP is therefore playing key roles both in neuronal development and synaptic plasticity. However, the molecular mechanisms underlying the functional regulation of FMRP-mediated mRNA trafficking, translation and subsequent protein synthesis are still largely unknown. My host laboratory has recently discovered that FMRP is sumoylated in vivo. Sumoylation is an essential post-translational modification that consists in the covalent conjugation of the protein SUMO (Small Ubiquitin like MOdifier) to specific lysine residues of target proteins. To start unraveling the functional consequences of FMRP sumoylation, I first compared the spine morphology of WT and FMRP KO neurones (Note that Fmr1 KO mice recapitulate the human disease). Morphological analyses of fmr1-KO neurons expressing the WT form of FMRP restores the correct mature spine morphology whereas expressing the non-sumoylatable form of the protein failed to do so. Moreover this non-sumoylatable FMRP acts as a dominant negative on WT neurons further confirming the important role of FMRP sumoylation in its regulatory function. We report here that FMRP sumoylation is required for the control of spine morphology.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