Programme final et livre des résumés

JED 2021

17-18 Mai 2021 - En ligne

XXIIIème Journées de l'Ecole Doctorale Normande de Biologie Intégrative, Santé, Environnement

Programme des XXIII^{ème} Journées de l'École Doctorale Normande de Biologie Intégrative, Santé, Environnement

Lundi 17 Mai 2021

09h00 – 09h30 : Ouverture de la XXIII^{ème} édition des JED nBISE :

- *Discours de **François Dauphin**, Directeur de l'École Doctorale nBISE*
- *Discours du Comité d'Organisation*
- *Diffusion de la vidéo de présentation du Laboratoire de Microbiologie, Signaux et Microenvironnement (LMSM) d'Évreux*

09h30 – 11h00 : Communications orales – Session 1 (6)

11h00 – 11h15 : Pause-café

11h15 – 12h00 : Conférence plénière :

- ***Pr. Pierre Déchelotte** : « Le microbiote intestinal, notre meilleur ami ! »*

12h00 – 12h45 : Déjeuner

12h45 – 13h45 : Session poster (9)

13h45 – 15h15 : Communications orales – Session 2 (6)

15h15 – 15h30 : Pause-café

15h30 – 16h30 : Table ronde sur le thème de « l'après-thèse » **:**

- ***Dr. Claire Grelle** : Responsable Enseignement Supérieur Recherche et Innovation à Évreux Portes de Normandie*
- ***Dr. Karim Atmani** : Chargé de Projets Scientifiques à la Division des Programmes d'Investissements de l'État à l'ANR*
- ***Dr. Bérénice Le Dieu-Lugon** : Chargée de Projet chez Sanofi*
- ***Dr. Ludovic Bretin** : Post-Doctorant à l'Institut de Chimie de Leiden aux Pays-Bas*

16h30 – 17h00 : Thesis project – Session 1 (6)

Programme des XXIII[ème] Journées de l'École Doctorale Normande de Biologie Intégrative, Santé, Environnement

Mardi 18 Mai 2021

09h00 – 09h30 : Thesis project – Session 2 (6)

09h30 – 09h45 : Discours de Praxens et N2S :

- ***Mme Nadine Picard*** *: Directrice de Praxens*
- ***Dr. Ségolène Depayras*** *: Chargée de Projets et Développement chez Praxens*
- ***Dr. Esther Le Toquin*** *: R&D Project Manager chez N2S*

09h45 – 12h00 : Conférences plénières :

- ***Dr. Isabelle Trinsoutrot-Gattin*** *: présentation sur les microorganismes de l'environnement*
- ***Pr. Marc Feuilloley*** *: présentation sur le microbiote cutané*

12h00 – 12h45 : Déjeuner

12h45 – 14h30 : Communications orales – Session 3 (7)

14h30 – 15h30 : Session poster (8)

15h30 – 17h15 : Communications orales – Session 4 (7)

17h15 – 17h45 : Vote du jury et du publique pour la remise des prix, clôture des JED

PROGRAMME COMMUNICATIONS ORALES

Lundi 17 Mai 2021

Session 1

Horaires	Participants
09h30 – 09h45	**Charlotte Toustou** *Improvement of monoclonal antibodies production in the diatom Phaeodactylum tricornutum by using new promoters*
09h45 – 10h00	**Fanny Potzeha** *Pro-coagulant monocyte-derived microparticles: a promising treatment to stop bleeding in a murine model of haemorrhagic stroke*
10h00 – 10h15	**Ivana Dabaj** *Muscle metabolic remodelling patterns in Duchenne muscular dystrophy revealed by ultra-high-resolution mass spectrometry imaging*
10h15 – 10h30	**Michael Alasoadura** *Peri-infarct low gamma activity correlates with recovery of dexterous forepaw function after experimental stroke*
10h30 – 10h45	**Mohammad Ahmad** *Development of high throughput approaches to identify cancer associated long non-coding RNAs*
10h45 – 11h00	**Oumayma Chkili** *Size-fractioned primary production and carbon pathway transfer in the Gulf of Gabes (Southeastern Mediterranean): influence of environmental conditions*

Session 2

Horaires	Participants
13h45 – 14h00	**Emmanuelle Carpentier** *Selenoprotein T: an antioxidant enzyme required for neuronal migration during corticogenesis*
14h00 – 14h15	**Aurélie Cullier** *Functionalized nanohydrogels decrease inflammatory and cartilage degradation biomarkers in an organoid model of horse cartilage tissue*
14h15 – 14h30	**Pierre Ledormand** *Response of a Liquorilactobacillus mali strain from cider to a lytic phage infection*
14h30 – 14h45	**Violaine Martin De Lagarde** *Evaluation of the oxidative potential of pyrotechnic smoke particles and in vitro toxic assessment on a 3D model of primary pulmonary cells at air-liquid interface*
14h45 – 15h00	**Thibault Chautrand** *Exposure of Pseudomonas fluorescens to gaseous NO_2 leads to multiple envelope alterations*
15h00 – 15h15	**Léon Serre-Fredj** *Coupling high frequency monitoring and bioassay experiments to investigate a harmful algal bloom in the Bay of Seine (French-English Channel)*

Improvement of monoclonal antibodies production in the diatom *Phaeodactylum tricornutum* by using new promoters

Charlotte Toustou*[1], Carole Plasson[1], Marie-Christine Kiefer-Meyer[2], and Muriel Bardor*[3,4]

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Monoclonal antibodies (mAbs) are booming through their uses as treatments against cancers and inflammatory diseases. Mainly produced in mammalian cells like CHO cells (Chinese Hamster Ovary cells), this kind of production presents some drawbacks (high costs, human-transmissible viral contamination...). So, new alternatives as plants (Lin *et al.,* 2018; Ma *et al.,* 2003) or microalgae are increasingly explored. Recently, mAbs directed against the Hepatitis B virus antigen (HBV) and the Marburg virus were produced in the diatom *Phaeodactylum tricornutum* (Hempel *et al.,* 2011; Hempel and Maier, 2012; Hempel *et al.,*2017), and in the case of the HBV, a biochemical and functional characterization was done (Vanier *et al.,* 2015 & 2018). However, although promising, the mAbs production by microalgae is still limited by some challenges, particularly on the improvement of production yield, which is for now, not sufficiently competitive to claim any industrial scale production. Thanks to a transcriptomic analysis of the diatom *P. tricornutum*, above fifty overexpressed genes induced by salt stress were found (Ovide *et al.,* 2018). With the aim of identifying "strong" promoters, bioinformatics analyses were performed on upstream regions of these genes. These analyses allowed us to confirm the presence of conserved pattern between the different potential promoter regions, but also to select thirty-three of them for further characterization. In order to validate them, these promoter regions were cloned in a plasmid vector upstream to the eGFP reporter gene, whose expression is measurable by fluorimetry. The overall goal being to select the two or three better promoter regions able to increase the production yield of mAbs in *P. tricornutum*.

Keywords: *Phaeodactylum tricornutum*, promotors, monoclonal antibodies

**Intervenant*

CO N°352441

Pro-coagulant monocyte-derived microparticles: a promissing treatment to stop bleeding in a murin model of hemorrhagic stroke

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Hemorrhagic strokes account for 20% of strokes and are due to a rupture of a blood vessel within the brain parenchyma (ICH). To date, no treatment exists for these ICH patients. Therapeutic studies based on administration of actors of coagulation pathway were conducted. However, the results have been shown ineffective. Targeted treatments are necessary to stop the induced bleeding. Therefore, microparticles (MPs) are interesting alternatives. Monocyte-derived MPs notably present the tissue factor (TF) bringing a pro-coagulant activity. The aim of this study is to generate exogenous pro-coagulant monocyte-derived MPs on a large scale, characterize them and study their efficacy as treatment in ICH murin model by collagenase injection. THP-1 were grown in a bioreactor and stimulated with TNF to generate pro-coagulant MPs. MPs were isolated and characterized by confocal microscopy and flow cytometry. Functional studies of MPs were performed by plasma clotting assays and TF+- western blot. Then, preclinical studies were assessed. Swiss mice received an intrastriatal injection of collagenase VII to mimic hemorrhage. 30 minutes after, MPs were injected as intravenous injection therapy. Hemorrhage volumes were quantified by MRI at 24h and neurological deficits were measured at 4h and 24h. Monocyte-derived MPs reduce from 30 minutes to 20 minutes the clotting time of human plasma in a dose-dependent manner. This was justified by the presence of TF on these MPs. A MPs dose response revealed that 1μg/g/mouse is the most relevant dose. Finally, the early i.v. injection of MPs significantly reduce of 43% the hematoma induced * and improve the neurological deficit of animals at 4h and 24h ** (* p< 0,001, n=15/group; **p> 0,01, n=15/group). We generated exogenous monocyte-derived MPs on a large scale. We highlighted their pro- coagulant role. These original data permitted to characterize their therapeutic potential in mice hemorrhagic stroke treatment. Moreover, this could be an outstanding target to develop new drugs to cure hemorrhagic stroke patients.

Keywords: Hemorragic stoke, microparticles, coagulation, bleeding.

Intervenant

Muscle metabolic remodelling patterns in Duchenne muscular dystrophy revealed by ultra-high-resolution mass spectrometry imaging

Ivana Dabaj*[1], Justine Ferrey, Florent Marguet , Vianney Gilard , Carole Basset , Youssef Bahri , Anne-Claire Brehin , Catherine Vanhulle , France Leturcq , Stéphane Marret , Annie Laquerrière , Isabelle Schmidt-Afonso , Carlos Afonso , Soumeya Bekri , and Abdellah Tebani

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Duchenne muscular dystrophy (DMD) is a common and severe X-linked myopathy, characterized by muscle degeneration due to altered or absent dystrophin. DMD has no effective cure, and the underlying molecular mechanisms remain incompletely understood. The aim of this study is to investigate the metabolic changes in DMD using mass spectrometry-based imaging. Nine human muscle biopsies from DMD patients and nine muscle biopsies from control individuals were subjected to untargeted MSI using matrix-assisted laser desorption/ionization Fourier-transform ion cyclotron resonance mass spectrometry. Both univariate and pattern recognition techniques have been used for data analysis. This study revealed significant changes in 34 keys metabolites. Seven metabolites were decreased in the Duchenne biopsies compared to control biopsies including adenosine triphosphate, and glycerophosphocholine. The other 27 metabolites were increased in the Duchenne biopsies, including sphingomyelin, phosphatidylcholines, phosphatidic acids and phosphatidylserines. Most of these dysregulated metabolites are tightly related to energy and phospholipid metabolism. This study revealed a deep metabolic remodelling in phospholipids and energy metabolism in DMD. This systems based approach enabled exploring the metabolism in DMD in an unprecedented holistic and unbiased manner with hypothesis-free strategies.

Keywords: omics, neuromuscular disorders, metabolomics, Duchenne muscular dystrophy, mass spectrometry, based imaging, muscle biopsy

Intervenant

CO N°354489

Peri-infarct low gamma activity correlates with recovery of dexterous forepaw function after experimental stroke

Michael Alasoadura*[1], Mahmoud Hazime[1], Pascale Quilichini[2], Jérôme Leprince[1], David Vaudry[1], and Julien Chuquet[1]

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In the chronic phase following brain ischemia, recovery of lost functions involves remodeling of cortical neuronal networks adjacent to the necrotic lesion. It has been shown that the perilesional region exhibits reduced excitability accounting for sub-optimal functional recovery. Furthermore, this dampened excitability coincides with a deficit in low gamma frequency oscillations (30-50Hz) of the local field potential. This gamma rhythm is used to synchronize neuronal activity in order to organize information processing and plasticity. However, the possible link between these changes and spontaneous functional recovery, albeit subpar, remains elusive. To propose new therapies aimed at correcting this hypo-excitability, it is thus crucial to further understand the relationship between excitability and recovery. To this end, we probed *in vivo*, the spontaneous neuronal firing and local field potential activity in the primary-somatosensory-front limb area corresponding to the peri-infarct cortex of mice subjected to a focal cortical ischemia at 7- and 21-days post stroke. In parallel, we evaluated the recovery of dexterous forepaw function in these mice. Neuronal spiking was found to be depressed in the peri-infarct zone at 7 days after stroke. Interestingly, recovery of skilled forepaw use was positively correlated with low gamma power at 7 days after ischemia, but this correlation weakens at 21 days after ischemia. Overall, our observations are consistent with the concept that correcting the excitability of the cortex surrounding a cortical lesion in the recovery phase of stroke is a relevant intervention to boost synaptic plasticity and facilitate functional recovery.

Keywords: Cerebral ischemia, Periinfarct cortex, Functional recovery, Local field potential, Neuronal spiking

*Intervenant

CO N°354455

Development of high throughput approaches to identify cancer associated long non-coding RNAs

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Ovarian cancer (OC) is the main cause of death from gynecological malignancies with emergence of 240,000 new cases and 130,000 deaths annually along with poor 5-year survival. OC is characterized by diagnosis at late stages (III/IV FIGO), and recurrence associated resistance to platinum-based chemotherapy. It is important to identify the molecular mechanisms involved in chemoresistance of this disease. Long non-coding RNAs (LncRNAs) have been identified in regulation of many biological processes and their perturbed expression is associated with progression of many diseases including cancer. In OC, despite a limited number of studies implicating LncRNAs, it appears clearly that they play important roles in many aspects of this disease, including response to treatment. Therefore, identification of new biomarkers predictive in the response to treatment and new therapeutic targets are considered as priority to improve patient's care. We studied the transcriptomic profiles of 50 tumor samples of patients who bear a homogenous disease of high grade and late stage serous OC by next generation sequencing (NGS), 30 showing complete response (RC) after 1st line chemotherapy and 20 partial response or stable disease (RI). We identified 250 differentially expressed genes (DEGs) between RC and RI, among which 97 lncRNAs. We selected 7 lncRNAs as candidates to explore the consequences of their inhibition in OC cell lines. Out of them, the downregulation of MYO16-AS1 by specific siRNAs appears to reduce the wound healing capacity of SKOV3 cells. We will further characterize the functions of MYO16-AS1 and the determinants of its action. Moreover, we will explore the possible role in the response to treatment of all the 97 differentially expressed lncRNAs, by using a CRISPR/Cas9-based functional screening, in order to get a comprehensive overview of all potentially involved lncRNAs in the response to treatment of OC cells. To that end we already established a SKOV3-derived cell line expressing CRISPR/Cas9 in an inducible way.

Keywords: Ovarian Cancer, LncRNAs, Next generation sequencing, chemoresistance, CRISPR/Cas9

Intervenant

CO N°353346

Size-fractioned primary production and carbon pathway transfer in the Gulf of Gabes (Southeastern Mediterranean): influence of environmental conditions

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There is little information on the primary production and the carbon pathway transfer in the Gulf of Gabes (Southeastern Mediterranean), despite this ecosystem is known to be highly productive and is under strong hydrodynamic and nutrient control. This study investigated the size structure of phytoplankton and production as well as the grazing impact of protozooplankton (< 200μm) and metazooplankton (> 200μm). Sampling was conducted in autumn in four stations localized from the north to the south of the Gulf and characterized by various hydrological and environmental conditions. Dilution experiments were carried out at each station to determine the growth rates of phytoplankton (pico (< 2μm), nano (2-10μm) and microcells (> 10μm)) and the rates of protozooplankton grazing on them. The grazing of metazooplancton on phytoplankton was also measured at each station, using the gut fluorescence content method. The nutrient availability of the Gulf had translated to high primary production and chlorophyll concentrations. However, there was a clear spatial gradient of trophic statue, which seemed connected to the complex hydrodynamic circulation, with north-south increase of nutrient, phytoplankton biomass and production. There were also changes in size structure and composition of phytoplankton, leading to a marked dominance of picoalgae in the north, which decreased towards south, where large algae took over and dominated biomass and production. All this was followed by different grazing pressure of metazooplankton and protozooplankton across stations. At the northernmost station, characterized by lower nutrient availability, protozooplankton dominated by aloricate ciliates showed the highest microbivory, as they consumed high fraction of picoalgal production. In the other stations, protozoans relied on small and large algae but they exhibited pronounced herbivorous activity at the southernmost station, where the heterotrophic dinoflagellates and loricate ciliates exhibited important proliferation. The metazooplankton, mainly composed by copepods, displayed also increased concentration and grazing impact from the north to south. These results suggest that carbon would be channeling to higher consumers through various trophic pathway and with variable efficiency according to a north-south gradient. Data from this study will serve for future modelling in order to describe the structural and functional characteristic of planktonic food-web.

Keywords: Primary production, zooplankton grazing, dilution experiment, gut content pigment, environmental conditions, Gulf of Gabes

Intervenant

CO N°354378

Selenoprotein T: an antioxidant enzyme required for neuronal migration during corticogenesis

Emmanuelle Carpentier*[1], Lisa Brunet[1], Mathieu T Castex[1], Arnaud Arabo[2], Magalie Bénard[3], Marine Di Giovanni[3], Youssef Anouar[1], and Anthony Falluel-Morel[1]

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Corticogenesis encompasses the developmental mechanisms responsible for the formation of the neocortex, a brain structure implicated in cognitive function, such as language, decision-making or attention in mammals. Some developmental diseases that impact human health could be due to alterations in neurocortical migration, differentiation or synaptogenesis. Oxidative stress is one of the various insults that can affect corticogenesis, resulting from accumulation of reactive oxygen species. A particular class of antioxidant enzymes, named selenoproteins, protect cells by maintaining redox homeostasis. We focused our attention on selenoprotein T (SELENOT) a recently discovered selenoenzyme, whose conditional deletion in neural lineage leads to transient brain volume reduction during development and a hyperactive phenotype in adult KO mice. These observations raise the hypothesis that SELENOT could be necessary for the proper establishment of neuronal circuitry during corticogenesis. The first aim of this study was to determine the relationship between cortex development and SELENOT expression, from gestational day 12 (E12) to one-year-old in mice. These data showed that SELENOT messenger RNA and protein are expressed as early as E12 and that their levels continuously increase during mice life. In addition, distribution of the tran- script was analysed by RNAscope *in situ* hybridization which revealed that SELENOT is mostly expressed in proliferative and differentiating layers in embryonic mice. Based on these data, *in utero* electroporation was performed in order to knockdown SELENOT expression in neuronal progenitors during corticogenesis, at embryonic day 14.5. Seventy-two hours after electroporation, a deficit in cells successfully reaching the cortical plate was observed, as well as an altered polarity of these SELENOT-deficient migrating neuroblasts. These alterations are associated with increased apoptosis in neocortex. These results revealed the implication of SELENOT in neuronal migration during corticogenesis, and may thus contribute to the understanding of the molecular and cellular mechanisms underlying neurodevelopmental deficits.

Keywords: Selenoprotein T, Development, Corticogenesis, Neuronal migration, Selenoproteins.

*Intervenant

CO N°353799

Functionalized Nanohydrogels decrease Inflammatory and Cartilage Degradation Biomarkers in an organoid model of horse cartilage tissue

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Osteoarthritis (OA), a common degenerative disease characterized in humans by debilitating chronic pain and inflammation, is expected to become the fourth most important cause of disability worldwide by 2020. Horse has been considered and proved to be as one of the most relevant patient animal model to study OA based on the cellularity, structural, biochemical and mechanical properties of cartilage, but also on its ageing evolution. OA is responsible for 60% of lameness in horses and may lead to abrupt ends of racehorse's career. No curative treatment is available and symptomatic treatments commonly used suffer from a limited efficiency and frequently adverse effects. The aim of this project is to develop a nanomedicine therapeutic approach based on functionalized hydrogels that could improve mechanical functions, protect the cartilage against inflammation and degradation processes, and reduce pain. Several formulations and concentrations of hydrogels were studied *in vitro* to evaluate their biocompatibility and phenotypic effects on equine articular chondrocytes (eAC). Dedifferentiated eAC were cultured *in vitro* as organoids in type I/III collagens sponges under hypoxia for 7 days, and interleukin-1bêta (IL-1β) was added into the media to mimic an inflammatory environment. First, the hydrogels formulations did not have cytotoxic effects and did not alter the proliferation and the viability of chondrocytes. Then, the expression of hyaline cartilage markers was downregulated by IL-1β which concomitantly induced a strong induction of inflammatory and catabolic markers. Interestingly, the combination of two formulations of hydrogels decreased the expression of mRNA encoding several degradation markers, such as some metalloproteinases (*Mmp1*, *Mmp3* and *Mmp13*), agrecanase (*Adamts5*) and inflammatory markers (*Ptgs2* and *Inos*). Finally, this combination reduced the expression of type I collagen protein in our cartilaginous organoids.
The biocompatibility and effectiveness of these hydrogels' formulations on inflammation and degradation allow considering *in vivo* study in horses first, for tolerance assessment and thereafter in horses that spontaneously suffered from osteoarthritis. These results showed that functionalized hydrogels could be an innovative therapeutic strategy in the treatment of OA.

Keywords: Osteoarthritis, Horse, Hydrogels, Nanomedicine

*Intervenant

Response of a *Liquorilactobacillus mali* strain from cider to a lytic phage infection

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Bacterial communities of fermented foods are well known for their technological and health potentialities. Although many studies are dedicated to the microbial diversity and fluxes within fermented food matrices, phages-bacteria interactions are still poorly described in these ecosystems. This is especially true when it comes to cider, an alcoholic fermented ap- ple beverage. The current work aimed at studying the transcriptomic and proteomic response of the lactic acid bacterium (LAB) *Liquorilactobacillus mali* UCMA 16447 during the lytic infection by phage UCMA 21115, both originating from cider. A RNAseq approach was used to monitor the transcriptomic response of *L. mali* UCMA 16447 after fifteen minutes (T15) and after one hour (T60) of phage infection, a non-infected culture being used as a control. More than 100 and 200 genes appeared to be up or down regulated at T15 and at T60, respectively. Overall, genes involved in cell motility, translation, carbohydrates metabolism and signal transduction were down regulated, while genes implicated in nucleotide metabolism and in the control of DNA integrity were upregulated, and this phenomenon increased over time. The same trends were confirmed by the proteomic study. At T60, 29 proteins were differentially expressed following the infection by phage UCMA 21115. Proteins involved in energy production and nucleotide metabolism were upregulated, while those related to cell motility were downregulated. The current study is the first report regarding the impact of a lytic phage on a LAB isolated from a fermented beverage. Getting knowledge about host response to phage infection is crucial to better control and understand microbial population equilibria throughout fermentation processes. This will possibly contribute to guaranteeing the production of safe and sustainable foods in the future.

Keywords: Transcriptomic, Proteomic, Phage, Bacteria, Fermented beverages, Cider

*Intervenant

CO N°353361

Evaluation of the oxidative potential of pyrotechnic smoke particles and in vitro toxic assessment on a 3D model of primary pulmonary cells at Air-Liquid Interface

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Pyrotechnic smokes are widely used for civilian and military applications and smokes combustion induces intense particulates pollution episodes. There are multiple types of smokes with various initial compositions, but there is a lack of data on chemicals produced after combustion and their toxic effects. In this study, we evaluated the toxicity of particles from a red signaling smoke (RSS) and from an hexachloroethane-based obscuring smoke (HC-OS) by measuring their oxidative potential (OP) and by exposing a 3D model of primary human pulmonary cells (NHBE) grown at the Air-Liquid-Interface to suspended particles. OP was examined with the dithiothreitol (DTT) and the antioxidant (acid ascorbic (AA)) depletion assays. Cytotoxicity (MTT, cell cycle) and genes expression (RT-qPCR) were explored after 24h of exposure (RSS and HC- OS). Physicochemical characterization of particles revealed that particles ($<1\mu$m diameter) are capable to penetrate deep into the airways after inhalation. Furthermore, RSS particles were more organic (quinones and polycyclic aromatic hydrocarbons) and less metallic (12 mg/g) than HC-OS particles that were mainly metallic (133 mg/g) especially in Al and Fe. At 50 μg/cm2, results showed that DTT was significantly depleted by RSS (74%) and HC-OS (42%) particles but AA was only depleted by HC-OS (42%). Both particles werenot cytotoxic but genes expression was modified and depending on particles type. Particles from RSS (50 μg/cm2) but not from HC-OS significantly increased superoxide dismutase 1 and 2 and heme oxygenase-1 expression. Both particles significantly induced NADPH quinone oxidoreductase-1 and interleukine-8 expressions whereas the catalase expression was unchanged. Because of their different chemical composition, smoke particles produced many different oxidative reactive species, which can be detectable by the two OP assays. Indeed, the reaction of DTT assay is mainly associated with organic components, which may explain the higher depletion by RSS particles. On the contrary, AA depletion is generally attributed to metalscorresponding to HC-OS depletion. Our study demonstrated that NHBE exposure to different smoke particles triggered an adaptive antioxidant response and lead to inflammatory response. This study improves the knowledge of the toxicity of pyrotechnic mixtures likesmoke particles to assess human health risk.

Keywords: Smokes particles, oxidative potential, Air Liquid Interface exposure, primary human pulmonary cells, antioxidant and inflammatory responses.

Intervenant

Exposure of Pseudomonas fluorescens to gaseous NO2 leads to multiple envelope alterations

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Nitrogen oxide (NO) and nitrogen dioxide (NO2) are reactive nitrogen species (RNS) produced by fuel combustion processes used in transport and industry. RNS are very reactive molecules causing important environmental problems such as smog and acid rains. RNS reactivity makes them toxic for living organisms at high concentrations, and these molecules play an important role in the immune system. As a result, bacteria have developed resistance mechanisms to RNS. Bacteria are surrounded by an envelope, which is the first bacterial structure encountered by RNS. Bacterial envelope are comprised of one or two lipid membranes and a peptidoglycan layer. Studying the effects of RNS on bacterial envelopes could therefore give important insights in understanding the reactions of bacteria to environmental pollution. *Pseudomonas fluorescens* is a ubiquitous gram-negative bacteria present in environments as diverse as atmosphere and human lung microbiota. In this study, *P. fluorescens* strain MFAF76a was exposed to gaseous NO2 to investigate the envelope alterations induced by this molecule as well as the cell response. The strain MFAF76a was isolated from air and presents several virulence factors, making it an interesting experimental model for environmental pollution studies. Environmental pollution was simulated by exposing *P. fluorescens* strain MFAF76a to 45ppm NO2 for 2 hours. Various membrane parameters were then assessed. Membrane permeabilization and membrane fluidity were assessed respectively by flow cytometry and fluorescence anisotropy. Cell response was assessed by a proteomic study. Results show that the bacterial membrane and morphology are modified by gaseous NO2. Furthermore, multiple key proteins involved in the synthesis of various envelope components are affected by these conditions. These proteins are involved in the synthesis of lipopolysaccharides, peptidoglycan and fatty acids. Therefore, most of the key components of bacterial envelopes seem affected by exposure to NO2. However, additional researches are necessary to distinguish the modifications caused directly by NO2 and the modifications resulting from a cellular response.

Keywords: Nitrogen dioxide, *Pseudomonas fluorescens*, envelope, membrane

*Intervenant

CO N°354404

Coupling High frequency monitoring and bioassay experiments to investigate a Harmful algal bloom in the Bay of Seine (French-English Channel)

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Coastal ecosystems are increasingly threatened by eutrophication and dystrophy, which can lead to massive phytoplankton blooms in particular hydrologic contexts. In the Bay of Seine, phosphorus (P) inputs from the Seine estuary have been largely reduced in the last decade leading to high N/P ratio inputs. Sudden nutrient pulses can result in large-scale phytoplankton bloom events in this bay. We conducted detailed high-frequency investigations of phytoplankton dynamics using a smart buoy (called SMILE), deployed in the Bay of Seine, equipped with a wide range of sensors measuring temperature, oxygen, salinity, turbidity, PAR, nutrients (WIZ-Systea and OPUS-Trios sensors) and photosynthetic parameter (Fast Repetition Rate Fluorimeter). The full pattern of the bloom dominated by a HAB of a Dinoflagellate species, *Lepidodinium chlorophorum*, was characterised and showed that an inflow of warm enriched freshwater triggered the bloom event. A decline in the P concentration led to a marked increase in the N/P ratio thereby reducing photosynthetic parameters and causing the collapse of the bloom. In parallel, we performed a bioassay experiment using water sampled during the bloom event to characterise the effects of different nutrient inputs on the fate of the bloom. Five different enrichments (control, N, P, N+Si and N+P+Si) were applied. After 5 days, biomass, flow cytometry diversity, photosynthetic parameters, alkaline phosphatase activity (APA), transparent exopolymeric substance (TEP) and nutrients, were measured. Only the (N+P+Si) enrichment supported growth of the phytoplankton community including *L. chlorophorum*, indicating potential N and P co-limitation. High level of APA and TEP production identified under limited growth conditions was associated with unbalanced nutrient inputs. A bloom collapse of *L. chlorophorum* due to the unbalanced N/P ratio induce massive TEP production.

Keywords: *Lepidodinium chlorophorum*, eutrophication, FRRf, Transparent exopolymeric particles, flow cytometry

*Intervenant

CO N°353761

Mardi 18 Mai 2021

Session 3

Horaires	Participants
12h45 – 13h00	**Hemily Batista-Silva** *Acute in vitro non-genomic and genomic effects of bisphenol A on testicular energy metabolism in zebrafish*
13h00 – 13h15	**Marine Pottier** *Decreased susceptibility to a major component of disinfectant associated to human and equine Pseudomonas aeruginosa isolates*
13h15 – 13h30	**Marion Aubourg** *Iron-limitation stress response in Staphylococcus lugdunensis*
13h30 – 13h45	**Agalic Rodriguez-Duboc** *Therapeutic potential of neurotrophic factors on cellular and molecular alterations induced by Apnea of Prematurity in the murine cerebellum*
13h45 – 14h00	**Poppy Evenden** *DNA damage in lymphocytes of female farmers' samples measured using the high-throughput alkaline comet assay and the link with the development of cancer*
14h00 – 14h15	**Marion Rouge** *The implementation of an organotypic culture model of prepubertal rat testis in order to study the role and the mechanisms of action of estradiol in male gonad*
14h15 – 14h30	**Maëva Drouault** *Characterization of the effects of the mycotoxin deoxynivalenol on estrogen signalling in MCF-7 breast carcinoma cell line*

Session 4

Horaires	Participants
15h15 – 15h30	**Mylène Verney** *Molecular detection of 7SL-derived small RNA is a promising alternative for trypanosomosis diagnosis*
15h30 – 15h45	**Renaud Parment** *Effect of panax quinquefolius and/or Vitamin C on 5-Fluorouracil chemotherapy-induced "fatigue", cognitive and emotional deficits in mice*
15h45 – 16h00	**Islem Hammami** *Identification of relevant argon binding sites within proteins*
16h00 – 16h15	**Hippolyte Paysant** *The pharmacomodulation of MIM1, an inhibitor of the anti-apoptotic protein Mcl-1, increases its specificity and selectivity: structure-activity relationship study*
16h15 – 16h30	**Lisa Poncet** *Mental Time Travel Abilities in the Common Cuttlefish*
16h30 – 16h45	**Mélanie Brosolo** *Prenatal alcohol exposure effects on oligo-vascular interactions in the developing cortex*
16h45 – 17h00	**Baptiste Lerosier** *Morphology of the superior temporal sulcus in schizophrenia patients: a marker of early or late environmental vulnerability to hallucination?*

Acute in vitro non-genomic and genomic effects of bisphenol A on testicular energy metabolism in zebrafish

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Introduction: Energy metabolism and the availability of energy substrates are indispensable factors for the correct performance of the spermatogenesis. However, aquatic *organisms,* especially fish, are very susceptible to exposure to xenobiotics, such as bisphenol A (BPA), which is closely related to the impairment of physiological and biochemical events that result in damages to spermatogenesis. Therefore, the aim of this study was to evaluate the acute *in vitro* non-genomic and genomic effects of BPA on biochemical changes of the testicular energy metabolism.

Methods: For this, testes from zebrafish (*Danio rerio*) were incubated with BPA (10 pM and 10 μM) for 1 hour to measure the content of lactate and glycogen, and to analyze the activities of lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Furthermore, through an organotypic culture system of testes from zebrafish incubated with BPA for 6 hours, the relative gene expressions of LDHBa, ALT2, glycogen phosphorylase (PYGL) and pyruvate carboxylase (PCXA) were analyzed by real-time reverse-transcription polymerase chain reaction (RT-qPCR).

Results: This study showed that 1 hour of incubation with BPA (10 pM and 10 μM) reduced the testicular content of lactate and glycogen, as well as the activities of LDH and ALT. In contrast, testicular AST activity was increased. In addition, the relative gene expressions of LDHBa, ALT2 and PCXA were up-regulated after 6 hours of incubation with BPA at 10 pM, whereas PYGL was no changed.

Conclusion: These results suggest that BPA impairs testicular energy metabolism and may compromise the supply of testicular energy substrates, which may impair the spermatogenesis.

Keywords: Xenobiotic, Danio rerio, testis, lactate

*Intervenant

CO N°354137

Decreased susceptibility to a major component of disinfectant associated to human and equine Pseudomonas aeruginosa isolates

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Background: Didecyldimethylammonium chloride (DDAC) is a quaternary ammonium compound used in several disinfection products in veterinary and hospital environments. In Human medicine, strains of *Pseudomonas aeruginosa* (PSAE) have been showed to present a decreased susceptibility to DDAC according to the NFEN-13727+A2 referential by incompletely understood mechanisms. Decreased susceptibility to antibiotics and disinfectant agents is a major concern to public health as it allows pathogens to persist in their environment. **Objectives:** The purpose of this study was to evaluate the existence of PSAE strains with DDAC decreased susceptibility among animals in Normandy, France. **Study design:** Based on a "One health" approach, our global project aims at a better understanding of the resistance mechanisms to disinfectants and antibiotics for PSAE. **Methods:** Between 1996 and 2020, 183 strains (Table I) isolated from animal samples (including 146 equines) were provided by our reference laboratory and routine diagnostic service. Minimum inhibitory concentrations (MICs) of DDAC were determined using broth microdilution method. Decreased susceptibility was defined as MIC≥64 mg/L, corresponding to the concentration of DDAC in a disinfectant solution according to the manufacturer's instructions. **Results:** During the 1996–2015 period all strain tested were sensitive to DDAC, whereas for years 2017, 2018, 2019 and 2020 respectively 20%; 26.3%; 6.5% and 12% of strains presented decrease susceptibility (Figure I). Over all 10.4% of the strains presented this phenotype and 84.21% of them were equine (Figure II). Finally, equine respiratory and genital samples are those who have shown the most decrease in sensitivity, with respectively 42.11% and 36.84% of all strains. **Main limitation:** From 1996 to 2015, only 23 strains have been isolated; for years 2017 and 2018, only strains showing antibimicrobial resistances phenotype were selected. **Conclusion:** Decreased susceptibility to DDAC is also present in the veterinary industry. Genomic analyses are underway to determine the relatedness of theses strains and to understand the mechanisms involved.

Keywords: Adaptation to a major disinfectant, component, quaternary ammonium, QACs, Pseudomonas aeruginosa, disinfectant, resistance, adaptation, One health, Medicine, Veterinary

Intervenant

CO N°354482

Iron-limitation stress response in Staphylococccus lugdunensis

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Staphylococcus lugdunensis is a coagulase-negative *Staphylococcus* that is part of the normal human skin flora but can cause serious infections similar to those generated by *Staphylococcus aureus*. Recognized as an emerging opportunistic pathogen, *S. lugdunensis* is responsible of acute endocarditis, skin and soft tissue infections, brain abscesses and osteoarticular infections. Because iron limitation is a crucial stress encountered during infectious process, we performed phenotypic study, globals proteomic and transcriptomic analysis of this species incubated with or whithout the iron chelator 2,2'-dipyridyl (DIP). We showedthat iron-limitation condition promoted biofilm formation and reduced the ability of *S. lugdunensis* to cope with oxidative stress (1 mM H2O2). In addition, cells of S. lugdunensis cultured with DIP were significantly less virulent than that grown under standard conditions in the larvae of *Galleria mellonella* model of infection. We verified that these phenotypes were due to an iron limitation by complementation experiments by addition of FeSO4. We characterized the first iron-limitation stress proteome and the first iron-limitation stimulon (by RNAseq approach) in *S. lugdunensis*. Among 1426 proteins identified, 349 polypeptides were differentially expressed under iron-limitation condition (222 more and 127 less abundant). Our data revealed that proteins involved in iron metabolism and carriers were over-expressed, as well as several ABC transporters and polypeptides linked to cell wall metabolism. Conversely, enzymes playing a role in the oxidative stress response (especially catalase) were repressed. Moreover, 175 genes were identified as members of the iron-limitation stimulon (127 up- and 48 down-regulated). Six gene-clusters known or likely required for iron acquisition were identified. Among them a new Energy-Coupling Factor type transporter (ECF), likely involved in iron captation, has been found to be strongly induced in *S. lugdunensis*. Moreover, genes involved in resistance to oxidative stress (including catalase), virulence, transcriptional regulation and hemin detoxification were also deregulated. These data provide some answers on the cellular response to the iron-limitation stress that is important for the opportunistic behavior of this pathogen. We concluded that this stress promoted biofilm formation, but decrease the oxidative stress resistance that may, at least in part, explain the reduced virulence of *S. lugdunensis* observed.

Keywords: Staphylococcus lugdunensis, iron, limitation, RNA, seq, proteomic, virulence.

*Intervenant

CO N°352601

Therapeutic potential of neurotrophic factors on cellular and molecular alterations induced by Apnea of Prematurity in the murine cerebellum

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Perinatal hypoxia is a leading cause of morbimortality worldwide. Up to 40% of newborns who experience oxygen deprivation suffer long term neurological impairment. The impact of a hypoxic brain injury has been well investigated; however, despite its functional importance and immaturity at birth, the involvement of the cerebellum remains poorly understood.
This work aims at shedding light on the mechanisms underlying cerebellar hypoxic injury. To this end, we developed an intermittent hypoxia (IH) protocol, consisting of repeated 2-minute cycles of hypoxia-reoxygenation (including 20 seconds at 5% O2) between P2 and P12 during 6 hours per day, which constitutes a valid model of Apnea of Prematurity (AoP). Histological studies following this protocol revealed a delay in cerebellar maturation post-IH. Moreover, compared to the controls, these mice presented long-term alterations in functions linked to the cerebellum such as learning and motor skills. Preliminary findings have shown that, after IH, genes involved in reactive oxygen species production are overexpressed while genes encoding antioxidant enzymes are underexpressed. These alterations suggest a failure of the defense system against ROS and could be responsible for neuronal death in the cerebellum. Based on these first results, we performed a transcriptomic study (by RT-qPCR) of genes involved in cell differentiation and migration. We analyzed the expression of these genes in different developmental stages (P4, P8, P12 and P21) and in the different cerebellar layers by using laser capture microdissection. This enabled us to pinpoint a potential timeframe and cell type of vulnerability; indeed, at P8, IH cerebella displayed the most downregulated genes, which was progressively reverted at later stages. Moreover, we identified several pathways involved in the pathophysiology of AoP such as “synapse formation” and “cytoskeletal scaffold” in neuronal layers and “myelination” in the white matter. This indicates that IH could modify the phenotype and activity of various cells and contribute to the observed histological and behavioral deficits.
The project provides elements to better understand the cellular and molecular aspects of AoP-induced cerebellar injury. In the long term, it could lead to the identification of novel therapeutic targets to address this socially and economically relevant health issue.

Keywords: Cerebellum, Apnea of Prematurity, Neurotrophic factors, Cell differentiation, Synaptic activity

*Intervenant

CO N°353995

DNA damage in lymphocytes of female farmers' samples measured using the high-throughput alkaline comet assay and the link with the development of cancer.

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Background: Many studies have shown the existence of a link between exposure to pesticides and a higher level of DNA damage. However, longitudinal studies are rare and associations between DNA damage measured with the COMET assay and the development of cancer is still largely unknown. Therefore, the aim of this study is to i) quantify DNA damage for the women included in a French agricultural cohort at two time points, ii) study the relation between DNA damage and agricultural tasks and iii) explore the link between DNA damage and cancer incidence. **Methods:** 320 female agricultural workers, issued from a representative sample of farms from a French department (Calvados), were enrolled in this study from 1997 to 2000 and completed a face-to-face questionnaire. 245 women gave a blood sample at enrolment (T0). Ten years later, 104 consented to give another sample (T10). Using the high-throughput COMET assay with an internal historical negative control, we were able to quantify DNA damage of PBMCs using a visual scoring system based on 200 nuclei scored in 4 categories. **Results:** Mean age of women was of 46 years. A majority of these females were farm owners or co-owners (n=135, 55%) that worked or participated in livestock tasks (n=159, 65%) and dairy production (n=109, 44%). The majority of females were exposed to pesticides through application of anti-parasites and insecticides to livestock (n=117, 48%) and disinfection of milking equipment (n=117, 48%), whereas very few applied pesticides to crops (n=3, 1.22%). At T0, those who carried out administrative (n=180, 73%) and domestic tasks (n=213, 87%) had a significantly lower damage score than those who were not involved in these tasks (p=0.0113 and p=0.0055, respectively). From enrolment to end of 2014, 29 incident cancer cases were diagnosed, of which 16 cases of breast cancer (55%). No association was found between DNA damage and cancer incidence. **Conclusion:** Consequences of occupational exposure that females undergo in the agricultural industry could be captured by the COMET damage score. Women's exposure to tasks indirectly related to the farm upkeep show a lower level of DNA damage than those not undertaking these tasks.

Keywords: COMET assay, Agriculture, Farming, Cancer

Intervenant

CO N°354454

The implementation of an organotypic culture model of prepubertal rat testis in order to study the role and the mechanisms of action of estradiol in male gonad

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The testis performs two major functions: spermatogenesis, which is the process of producing sperm and steroidogenesis, which is the process of synthesis of steroid hormones. Among steroids synthesized by testis, there is testosterone and estradiol. The bioconversion of androgens to estrogens depends on the activity of the cytochrome P450 aromatase enzyme. Nowadays, it is well established that estrogens play an important role in maintenance of testicular functions and therefore in male reproduction. However, if the effects are known *in vivo*, the mechanisms of action are not elucidated. Therefore, it was necessary to develop an organotypic culture model of prepubertal rat testis in order to study the mechanics of estrogens *in vitro*. To this end, testicular explants of 1mm3 of prepubertal rats were cultivated on inserts during 72 hours and expose the explants to the medium and gas throughoutthe time of the culture of incubation in an atmosphere of 32°C; 5% CO2. The quality of the explants at the end of the culture was evaluated according to histological and immunological analyses, but also by quantification of the expression of the specific genes for the different testicular cell populations by RT-qPCR (vimentin, Sertoli cells; Hsf2, round spermatids; Protamin 2, spermatids and spermatozoa). Culture media was collected at the end of the culture to measure concentrations of estradiol and testosterone by ELISA assay. Our results demonstrate a maintenance of the structure and spermatogenesis of testicular explants in culture and also a maintenance of steroidogenesis. This organotypic culture model allows us to initiate different treatments such as estradiol receptor inhibitor to antogonize estradiol effects on receptors and aromatase inhibitor to suppress the endogenous estradiol of the explants. This allows to study the importance of estradiol in testicular activities.

Keywords: Organotypique culture, estrogens, testis, spermatogenesis

*Intervenant

CO N°353845

Characterization of the effects of the mycotoxin deoxinivalenol on estrogen signalling in MCF-7 breast carcinoma cell line

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Mycotoxins are natural occurring toxicants, produced by the secondary metabolism of some fungal species developing on crops. Thus, mycotoxins contaminate food chain and constitute a global health issue because of their adverse effects on human health when ingested. deoxynivalenol (DON), also known as “vomitoxin” because of its emetic effects, is at once among the most widespread mycotoxins and at the same time the most concerning ones. DON is a potent inhibitor of protein synthesis, inducing cell death through its interaction with ribosomes. However, a few studies suggested the existence of direct or indirect effects of DON on estrogenic signalling. In order to characterize DON’s effects on estrogenic signalling, an *in vitro* approach was performed. MCF-7 cells (a breast carcinoma cell line which highly expressed the nuclear estrogen receptor ERα) were exposed to various concentrations of DON (10-6 M, 10-5 M, 10-4 M). First, a crystal violet assay was conducted to assess DON’s cytotoxic effect in MCF-7 cells. After a 72h exposure only the highest concentration of DON (10-4 M) induced a decrease in cell viability, the others concentrations were thus considered safe in regard to cytotoxicity. Then, estrogen-responsive genes (*TFF1*, *PR*, *CTGF*) expression was analysed by RT-qPCR. After a 6h exposure DON stimulated the expression of all the 3 genes, thus indicating its ability to activate estrogen signalling pathway. This positive effect of DON on estrogen-responsive genes expression was only partially reversed by tamoxifen, a non-steroidal antagonist of ERα, and by siRNA-mediated knock-down of ERα. Taken together, these results indicated that if direct binding of DON on ERα could not be excluded to explain DON’s effect on estrogen signalling, others mechanisms of action were likely to be involved. To further investigate this hypothesis, the phosphorylation status of ERα was assessed by Western blotting. After a 30 min exposure, DON slightly increased ERα phosphorylation at serine 118 site. Our study demonstrates that DON could indirectly activate estrogen signalling in MCF-7 cells by inducing ERα phosphorylation. Further investigations are needed to understand the mechanism by which DON induced ERα phosphorylation.

Keywords: Mycotoxin, Deoxinivalenol, Estrogen signalling

*Intervenant

CO N° 354558

Molecular detection of 7SL-derived small RNA is a promising alternative for trypanosomosis diagnosis

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Equine trypanosomosis comprises different parasitic diseases caused by protozoa of the subgenus *Trypanozoon*: *Trypanosoma equiperdum* (causative agent of dourine), *Trypanosoma brucei* (nagana) and *Trypanosoma evansi* (surra). Due to the absence of a vaccine and the lack of efficacy of the few available drugs, these diseases represent a major health and economic problem for international equine trade. Development of affordable, sensitive and specific diagnostic tests is therefore crucial to ensure the control of these diseases. In the last decades, cell-free mRNA raised a particular interest in the scientific community as diagnostic biomarkers, notably in oncology. In that way, it has been shown that a small RNA derived from the 7SL gene (7SL-sRNA) is produced in high concentrations in sera of cattle infected with different species of trypanosomes. Our objective was to determine whether 7SL-sRNA could serve as a marker of active infection in equids. Using negative sera from field and positive sera from mares experimentally infected with *T. equiperdum*, we analysed the sensitivity and the specificity of the 7SL-sRNA detection and the stability of the 7SL- sRNA. 7SL-sRNA were amplified with a *Trypanozoon*-specific double-step RT-qPCR. The 7SL-sRNA signal was detected prior to the seroconversion of the experimentally infected horses. There was a rapid loss of 7SL-sRNA one day post-trypanocide treatment of infected animals. The 7SL-sRNA RT-qPCR allowed an early detection of a treatment failure revealed by glucocorticoid-induced immunosuppression. The specificity of this assay was confirmedon 63 sera from horses seronegative for dourine. The 7SL-sRNA signal remained stable insera during at least 7 days of storage (at 4°C, room temperature or 30°C), suggesting that there is no need to refrigerate serum samples before analysis. Our findings demonstrate continual detection of 7SL-sRNA over an extended period of experimental infection, with signals detected more than six weeks after inoculation. The detection of a strong and consistent 7SL-sRNA signal even during subpatent parasitemia highlight the promising nature of thisnew diagnostic method of equine trypanosomoses.

Keywords: 7SL, sRNA, diagnosis, equine trypanosomosis, RT, qPCR, *Trypanosoma equiperdum*

*Intervenant

CO N°354183

Effect of Panax quinquefolius and/or Vitamin C on 5-Fluorouracil chemotherapy-induced “fatigue”, cognitive and emotional deficits in mice

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Background: Cognitive impairment and fatigue described in cancer patients treated with chemotherapy, having a negative impact on quality of life. Panax quinquefolius (PQ) was shown to be effective against fatigue in patients undergoing cancer treatment. Using a behavioral animal model, the impact of a solution of Qiseng (PQ + vitamin C (VC)) or VC was studied on cognitive, emotional and/or fatigue deficits induced by 5-Fluorouracil (5-FU) chemotherapy. **Methods**: C57Bl/6 mice received 3 intraperitoneal injections (*ip*) of 5-FU (60 mg/kg/week) or NaCl (0.9%) co-administered with 5 daily gavages for 2 or 3 weeks of placebo, VC (19 mg/kg) or Qiseng (140 mg/kg PQ + 19 mg/kg VC) from the 2nd week of 5-FU treatment. Behavioral tests evaluating activity/fatigue, anxiety or depression and spatial cognition were undertaken. Following treatments, evaluation of the levels of ginsenoside and pro-inflammatory cytokines in the blood, alterations in the gut microbiota and modifications in the intestine and brain were performed. **Results:** 5-FU significantly reduced locomotor/exploration activity, potentially associated with fatigue, caused spatial cognitive impairments, accompanied by a decrease in neurogenesis in the hippocampus (Hp). VC did not prevent the deleterious effects of 5-FU. However, Qiseng prevented the impact of chemotherapy on activity/fatigue and on neurogenesis only in the ventral Hp. Administration of Qiseng induced the presence of ginsenosides Rb1, Rc,Rd and metabolite protopanaxadiol in serum. Intestinal inflammation and tissue damage in 5-FU mice were attenuated by Qiseng. Chemotherapy deficits were coupled with increased caecum levels of *Lactobacillus*, compensated by Qiseng, which leaded to a decrease in *Proteobacteria* classes. 5-FU increased plasma pro-inflammatory cytokines, TNF-a, IL-2, IL-12, IL-17 prevented by VC and Qiseng, IL-6 and MCP-1 only compensated by Qiseng. In 5-FU brain, elevation of IL-6 receptor gp130 expression was observed, with a decrease in proliferation of neural stem cells in the Hp. Qiseng prevented partially gp130 induction and totally inhibition of proliferation in Hp. **Conclusion:** By the action of ginsenosides, Qiseng prevented symptoms of fatigue and reduced chemotherapy damage by preventing *Lactobacillus* elevation in caecum, blocking the increase of plasma IL-6 and MCP-1, and reducing gp130 expression in brain, thus protecting proliferation/neurogenesis in the ventral Hp.

Keywords: Panax quinquefolius, Vitamin C, Chemotherapy, Fatigue, Cognitive functions, IL, 6, MCP, 1

*Intervenant

Identification of relevant argon binding sites within proteins

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Argon (Ar) belongs to the group of chemically inert noble gases, which display a remarkable spectrum of clinically useful biological properties. Noble gases by binding to physiologically relevant sites in proteins involved, for example in cell signaling would induce functional modifications that could lead to interesting protective properties. However, the literature on this is relatively small and the fascinating biology of these atoms is still being uncovered.
In an attempt to better understand noble gases, notably argon's mechanism of action, we mined a massive molecular modelling database which lists all possible noble gas binding sites in the proteins listed in the Protein Data Bank. We developed a new method of analysis to identify amongst all predicted noble gas binding sites, the potentially relevant ones within protein families, which are likely to be modulated by Ar. Our method consists mainly in determining within structurally aligned proteins, the conserved binding sites whose shape, localization, hydrophobicity and binding energies are further examined. This method was applied to the analysis of three protein families where crystallographic noble gas binding sites are known. Our findings indicate that amongst the most conserved binding sites, either the most hydrophobic one or the site which has the best binding energy correspond to the crystallographic noble gas binding sites with the best occupancies, therefore the most affinity for the gas. This method will allow us to predict relevant noble gas (Ar) binding sites that would be endowed with potential pharmacological interest and thus Ar targets that will be prioritizedfor further studies including *in vitro* validation.

Keywords: Argon, noble gas, modelling, structural alignment, conservation rate, proteins targets

*Intervenant

CO N°354107

The pharmacomodulation of MIM1, an inhibitor of the anti-apoptotic protein Mcl-1, increases its specificity and selectivity: structure-activity relationship study

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Ovarian cancer is the leading cause of death from gynaecological cancers due to the development of chemoresistance. Protection against cell death by apoptosis is an essential part of this chemoresistance. Our laboratory's work has highlighted the cooperation of two anti-apoptotic proteins Bcl-xL and Mcl-1 in this phenomenon, their concomitant inhibition leading to the death of chemoresistant cells. In some cases, inhibition of Mcl-1 alone is sufficient to induce massive apoptosis. Inhibition of Mcl-1 in the clinic is still problematic and up to very recently, no in vivo active Mcl-1 inhibitor had been described. The MIM1 compound was identified by Cohen et al. in 2012 as a Mcl-1 inhibitor after screening more than 70,000 molecules and the study of its biological activity in vitro showed a cytotoxic activity on murine leukemia cells. On the other hand, it has shown only a modest effect on our ovarian tumor lines. Our multidisciplinary work has made it possible to correct the chemical structure of this compound and has shown that the pharmacomodulation of the MIM1 molecule enables a significantly increase of its inhibitory activity against Mcl-1. The structure-activity relation-ships associated with molecular modeling work have allowed us to identify key residues and structures responsible for this activity. In addition, we have also shown that the introduction of certain modifications has led to the production of bispecific molecules for Mcl-1 and Bcl-xL. We now use this information to design completely original molecules that may have strong activity and excellent selectivity for Mcl-1.

Keywords: Cancer, ovarian, apoptosis, medicinal chemistry.

Intervenant

CO N°352840

Mental Time Travel Abilities in the Common Cuttlefish

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Mental Time Travel (MTT) is the ability to remember and reexperience personal past events (i.e. episodic memory), and to anticipate and pre-experience personal future events (i.e. episodic future thinking). Some researchers hypothesised that it is a human-specific capacity, and that animals, unable to escape their present state, cannot travel back and forth through their mind. However, evidences over the recent years coming from scrub jays and primates suggest that they possess complex past and future thinking. Studying animals brings valuable insights to understand the evolution of MTT, but focusing on phylogenetically-close species restricts our ability to disentangle evolutionary drivers as their complex cognitive abilities might have evolved from a common ancestor. Among invertebrates, cephalopods, such as the common cuttlefish *Sepia officinalis,* are known for their incredibly complex cognition, even though they evolved separately from vertebrates for 500 million years. Cephalopods are thus excellent candidates for the study of MTT. Over the last years, common cuttlefish were shown to possess episodic-like memory, source memory, self-control, flexible and future-oriented foraging behaviour, and future planning abilities. These abilities correspond to different behavioural aspects of retrospective and prospective MTT. Cuttlefish show us that these behaviours are not restricted to higher vertebrates or humans, but probably a lot more widespread than previously thought.

Keywords: Cognition, Mental Time Travel, Cuttlefish, Memory, Future planning, Cephalopods

*Intervenant

CO N°354061

Prenatal alcohol exposure effects on oligo-vascular interactions in the developing cortex

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Aim: Alcohol consumption during pregnancy can cause fetal alcohol syndrome (FAS), referring to the most severe expression of fetal alcohol spectrum disorder (FASD). However, most FASD children do not present the characteristic dysmorphism feature of FAS whereas they are not devoid of brain impairments and behavioral troubles which are frequently detected with schooling. Consequently, these children escape from an early diagnosis and several years of care are lost. Recent data from the laboratory obtained in both mouse and human have shown that prenatal alcohol exposure (PAE) alters the development of the brain vasculature causing a loss of the radial orientation of cortical microvessels. At a mechanistic level, a tight communication between cortical vessels and oligodendrocytes has been revealed. Indeed, it is now demonstrated that during neurodevelopment, the migration of oligodendrocytes is vasculo-dependent. **Working hypothesis:** Altogether these data support that *(i)* the deleterious effects of alcohol on brain vasculature would impair the developmental profile of oligodendrocytes and consequently *(ii)* white matter organization. **Objectives**: *(i)* to research the effects of alcohol on the expression of differentiation markers of the oligodendrocyte lineage, *(ii)* to perform a morphometric characterization of the positioning of oligodendrocytes in the developing cortex by targeting vascular interactions, and *(iii)* to investigate white matter consequences. **Results**: Experiments revealed a close interaction of migrating oligodendrocytes with radial microvessels of the developing cortex. At a mechanistic level, Western blot experiments performed at several developmental stages (GD20, P2 and P15) indicated that prenatal alcohol exposure impaired the oligodendrocyte lineage by targeting immature and mature oligodendrocytes. At a morphometric level, PAE altered the cortical positioning of Olig2- positive cells and induced a white matter disorganization. **Conclusion:** These results argue in favor of an anchored oligo-vascular communication involved in the cell fate of oligodendrocytes. Vascular defects induced by prenatal alcohol exposure would impairs oligodendrocytes positioning and consequently white matter and cortical organizations. They support that a microvascular dysfunction would contribute in the behavioral troubles associated to FASD.

This work is supported by Fondation Paralysie Cérébrale, Fondation de France, FEDER3R, ANR and Normandy Valorisation. MB is a recipient of a fellowship from the French Research ministry.

Keywords: Alcohol, Oligodendrocytes, Vessels

*Intervenant

Morphology of the superior temporal sulcus in schizophrenia patients: a marker of early or late environmental vulnerability to hallucination?

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Auditory verbal hallucinations (AVHs) are one of the most common and debilitating symptoms in schizophrenia, and appear to involve the superior temporal sulcus (STS). Sulcus patterns may reflect early vulnerability, probably genetic. In contrast, cortical thickness is dynamic and variable during development, and may reflect the impact of environmental factors on brain maturation. In this study, we aimed to determine whether AVHs were associated with STS morphological changes and whether these changes involved sulcal patterns or cortical thickness. Our study included 73 schizophrenia patients (DSM-IV), of whom 53 were prone to AVHs (AVH+) and 20 lacked AVHs (AVH–), as well as 100 healthy control volunteers (HC). All participants underwent a 3T MRI brain scan. We examined the cortical thickness and sulcal pit differences among the three groups, and how these factors were asso- ciated with AVH severity. Compared to HC, AVH+ patients showed a significantly reduced cortical thickness in the left temporal and frontal regions, and especially in the STS ($p \leq 0.05$, FDR corrected). Cortical thickness in the central part of the left STS was negatively correlated with the auditory hallucination rating scale score ($R = -0.50$; $r^2 = 0.25$; $p = 0.01$,uncorrected). Compared to HC, AVH+ patients exhibited a different distribution of the number of pits in the left STS, suggesting a less complex morphological pattern ($p = 0.05$). These results support the hypothesis that auditory hallucinations are related to a particularSTS morphology that might be due to both early and late factors.

Keywords: Schizophrenia, Auditory verbal hallucination, MRI, Sulcus Morphology

Intervenant

CO N°354483

PROGRAMME THESIS PROJECTS

Lundi 17 Mai 2021

Session 1

Horaires	Participants
16h30 – 16h35	**Vincent Camus** *Multiparametric characterization of primary mediastinal large B-cell lymphoma*
16h35 – 16h40	**Mélanie Fortier** *Pisum sativum root development under water deficit: involvement of Root Extracellular Trap (RET) and exudates?*
16h40 – 16h45	**Caroline Lahogue** *Treatment strategy targeting 5-HT6R in an innovative animal model of schizophrenia*
16h45 – 16h50	**Lucie Martin** *Physical constraints during chemotactic migration of glioma: nuclear alterations and potential genetic instability*
16h50 – 16h55	**Alexia Gaudry** *Root immunity: the role of root cap and its secretions in plant protection*
16h55 – 17h00	**Rima Salma** *Studies of biological pathways involved in radioresistant of cancer stem cells after hadron irradiation*

Mardi 18 Mai 2021

Session 2

Horaires	Participants
09h00 – 09h05	**Emma Vidal** *Cartilage engineering using vegetal biomaterials*
09h05 – 09h10	**Lucie Prevost** *Functional interaction between Angiotensin II type 1 receptor (AT1) and Urotensin II receptor (UT)*
09h10 – 09h15	**Marie Deschler** *NEMESIS: Interactions and chemical communication between toxic diatoms Pseudo-nitzschia and primary consumers (Copepods)*
09h15 – 09h20	**Laura Moutard** *Steroidogenesis and androgen/estrogen signalling pathways in in vitro matured testicular tissues of prepubertal mice*
09h20 – 09h25	**Céleste Nicola** *Impact of cancer and check-point inhibitors immunotherapies on emotional reactivity and cognitive functions in mice*
09h25 – 09h30	**Giulia Binarelli** *Computerized intervention for the improvement of cognitive difficulties in women treated for breast cancer: implementation, follow-up and evaluation of the Cog-Stim research program*

Multiparametric characterization of primary mediastinal large B-cell lymphoma

Vincent Camus*[1,2], Alexandra Traverse-Glehen[3], Fanny Drieux[4], Elena-Liana Veresezan[5], Clementine Sarkozy[6], Elodie Bohers[2], Philippe Ruminy[2], Mathieu Viennot[2], Pierre-Julien Viailly[2], Pierre Decazes[7], Stephanie Becker[7], David Tonnelet[7], Hervé Tilly[1], Thierry Jo Molina[8], and Fabrice Jardin[1,2]

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Primary Mediastinal Large B-cell lymphoma (PMBL) is a rare entity, accounting for 3- 5% of all non-Hodgkin lymphoma (NHL) and distinct from diffuse large B-cell lymphoma (DLBCL). PMBL occurs mostly in young female adults (median age 35 years old) with large mediastinal mass unlike most other aggressive NHL. Histologically, making the diagnosis can be challenging due to wide morphological variations and no pathognomonic marker. Gene expression profiling (GEP) studies have identified a very distinct molecular signature between PMBL and DLBCL, notably with *JAK2*, *PDL1* and *PDL2* overexpression. Preliminary data supports the hypothesis of an immune escape relying on genomic rearrangements, as a main driver for PMBL pathogenesis. Clinical data demonstrated excellent results for patients treated with dose-dense immunochemotherapy based on rituximab and anthracyclines. However, approximately 15% of patients are primary refractory (PR) and those patients will almost never respond to subsequent chemotherapy. Early identification of PR patients in order to direct them towards anti-PD1 / PDL1 immunotherapy is a major challenge to improve their prognosis. The objectives of the present work are as follows: (i) establish a GEP signature capable of predicting increased sensitivity to anti PD1 immunotherapy; (ii) characterize the mutational profile and tumor microenvironment (MET) of PMBLs; (iii) assess circulating tumor DNA release and clonal evolution to improve patient follow-up; (iv) describe body composition evaluated by computed tomography and impact on patients' outcome; (v) define a multi-parametric biological basis for the introduction of immunotherapy as 1st line treatment in combination to chemotherapy. Two PMBL cohorts will be analyzed: (i) a retrospective multicentric LYSA cohort that enrolled 313 patients treated with first-line R-CHOP/R-ACVBP between 2007 and 2017 with available PET imaging and FFPE and/or frozen biopsies. Immunohistochemistry, FISH, RT-MLPA and Next-Generation Sequencing (NGS) analysis are planned; (ii) a prospective cohort (*NCT04824950*) activated in 2021 in LYSA centers dedicated to ctDNA monitoring during first-line R-CHOP/R-ACVBP treatment. It is expected to enroll 87 patients in 4 years with ctDNA collection at baseline, post 2 and 4 courses of chemotherapy and in caseof relapse. Molecular response will be evaluated by NGS with a restricted 52 gene panels and compared to PET results.

Keywords: primary mediastinal Large B cell lymphoma, tumor microenvironment, gene expression profile, molecular signature, circulating tumor DNA, MRD

Intervenant

Pisum sativum root development under water deficit: involvement of Root Extracellular Trap (RET) and exudates?

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The consumption of vegetable proteins is increasingly widespread. In this context, legume crops such as Pea (*Pisum sativum*) are interesting for the nutritional quality of their seeds. However, drought is a major threat for these crops resulting in severe losses. The below-ground root system and their secretions are essential to cope with water stress [1]. To promote pea crop development, a better understanding of its root system responses to water deficit is crucial. To this end, we developed a Nuclear Magnetic Resonance (NMR) metabolomic approach to explore in depth root exudation profiles under water stress. Furthermore, at the root tip, the Root Extracellular Trap (RET) also contributes to the resilience of the plant from environmental stresses [2,3]. The RET is composed of specific cells, called "associated cap-derived cells" (AC-DCs) embedded in a thick mucilage containing metabolites and glycopolymers [2,4]. The RET plays a key role in interactions in root protection against biotic stresses [5]. We hypothesize that the RET is also involved in root protection against drought [4]. Here, we investigate the involvement of three types of glycopolymers, which are potential candidates in the RET to play a role against water deficit: arabinogalactan proteins (AGPs), extensins and pectins [4,6,7]. Mutants of the model plant *Arabidopsis thaliana*, affected in the production of these cell wall polymers, will also be used. Pea seedlings are sown in agar medium; the water deficit is simulated using polyethylene glycol (PEG). Biochemical and cell imaging approaches are used to characterize the production and release of AC-DCs, AGPs, extensins and pectins under water deficit conditions. Interestingly our preliminary results show that moderate water stress increases root growth and RET production.

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Keywords: arabinogalactan proteins (AGPs), extensins, NMR spectroscopy, pectins, Pisum sativum, Root Extracellular Trap (RET), root exudates, water deficit

*Intervenant

TP N°354087

Treatment strategy targeting 5-HT6R in an innovative animal model of schizophrenia

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Schizophrenia is the most frequent psychosis affecting 1% of worldwide population. Schizophrenicpatients are treated with antipsychotic drugs which have a symptomatic effect on positive symptoms (delirium, hallucination...), but not on the negative (avolition, anhedonia, sensory gating deficits...) and cognitive symptoms (memory impairment...) which remain hardly attainable. Targeting 5-HT6R receptors, known to be involved in cognitive processes could improve currents treatments. Indeed, blockade of 5-HT6R has a positive effect on memory deficits in rodent models. Therefore, we will investigate the effect of one of the most selective 5-HT6 antagonist (SB-271046) to alleviate cognitive functions alterations. We aim at testing this strategy in an innovative 2-hit animal model (genetic x environmental factors), combining the deletion of serine racemase (SRKO mice) associated with a maternal separation for 24h at post-natal day 9 (early stressing environmental factor), which both have been previously suggested to induce schizophrenia-like symptoms. The first part of the work will consist in characterizing the 2-hit mouse model. While first data revealed behavioural deficits (decreased social behaviour, sensory gating deficits, hyperactivity) compared to 1-hit (1-hit SRKO or 1-hit maternal separation), memory performances need to be assessed to complete the cognitive profile, and particularly spatial and recognition memory. This will be done with a paired-associated learning task (touch screen). An ex vivo electrophysiological study will be thereafter performed to investigate functional plasticity at hippocampal synapses and possible deregulation. The second part will be the assessment of SB-271046 effects on the 2-hit model using the same experimental techniques. Thus, our work aims to contribute to the development of a new innovative animal model of schizophrenia, and to underline the interest of targeting 5-HT6R to treat the cognitive and negative symptoms of the disease.

Keywords: Schizophrenia, Serotonin type 6 receptor, Gene, Environment interaction, Animal model, memory, Cognition

Intervenant

TP N°354192

Physical constraints during chemotactic migration of glioma: nuclear alterations and potential genetic instability

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Glioblastoma multiforme (GB) is the most common and aggressive primary tumor of the central nervous system. Despite aggressive treatment consisting of chirurgical resection, radiation and chemotherapy, the median survival is only around two years. This poor prognosis is partly explained by the invasiveness within the brain and resistance to treatments. Glioma cells respond to chemotactic signals by exploiting the network of microvascular pathways and white matter tracts. These migration routes are likely to involve various mechanical and physical constraints. The loss of nuclear envelope (NE) integrity induced by reduced Lamin A/C expression in solid/rigid tissue, may promote DNA damage by diffusion of DNA repair proteins, thus potentially leading to molecular heterogeneity, recurrence and treatment resistance. Analyses of The Cancer Genome Atlas and IVY-glioblastoma atlas project databases were here performed to evaluate the variable expression, in the different glioma molecular sub- types, of NE and DNA gene expression levels. To sum up, we showed a differential expression of NE proteins such as Lamins A/C, B1 and B2, and of DNA breaks repair proteins between gliomas, within GB, and among specific GB areas. Immunohistochemical experiments from human GB samples confirmed that Lamin A/C was differentially expressed among GBs, and that Lamin B1 expression was associated with a reduced expression of Ku80 in cellnuclei within tumor bulk. On adherent glioma cell lines, and patient derived-glioma cells, we observed that increased cell density and tumor mass induced a decreased expression in Lamin A/C expression and Lamin B1, events associated with differential occurrence of nuclear 53BP1-positive foci and of heterochromatin markers (H3K9me2,3). We currently develop *i)* GB organoids obtained from GB fresh samples thus maintaining the cellular heterogeneity of parent tumor, *ii)* migration of glioma cells under microfluidic systems and chambers mimicking physical constraints and *iii)* in matrices (HCS Pharma, Lille) to test different elastic modulus for 3D cell culture of GB cells, in which we aim mimicking a microenvironment with the constraints of human brain tissues. Together, we hope to obtain new information on the link between physical constrains, pressure on cell nuclei, and genetic and epigenetic regulation during tumor cell migration.

Keywords: glioblastoma, physical constrains, genetic instability, nuclear envelope, DNA repair

*Intervenant

TP N°354481

Root immunity: the role of root cap and its secretions in plant protection

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The root is an underground organ of central importance to plant development. It provides an anchor to the plant in soil, ensures its feeding and contributes to its protection. The root cap, releases cells named border cells (BCs) into the rhizosphere. Recent studies have shown that BCs produce a mucilage composed of polysaccharides, proteins, secondary metabolites and extracellular DNA (exDNA). The BCs and mucilage form the so-called "Root Extracellular Trap" (RET) a structure that is analogous to the Neutrophil Extracellular Trap (NET) found in mammals. The RET allows communication between the rhizosphere, underground microorganisms and the plant. The PhD thesis work of Dr. M. Ropitaux (2015-2018) on soybean (*Glycine max*) root in our laboratory has allowed the emergence of new hypotheses. Therefore, xyloglucan, mannan and exDNA, three polymers found in the RET of soybean (*Glycine max*), are suggested to play a role in RET structural integrity and root immunity. First, by using confocal microscopy, cytochemical and immunocytochemical approaches, we have checked that the root cap of the new varieties of soybean (*Glycine max*) and pea (*Pisum sativum*) release a RET and the polymers previously mentioned. At the same time, enzymatic treatments with DNase I were performed in soybean to degrade exDNA and examine whether the integrity of the RET is modified. Other experimentations will be carried out in order to study the role of the polymers in maintaining the RET integrity by a specific deconstruction of the RET polymers or by using bacteria. The composition and structure of RET polysaccharides and the root cap will be analyzed using chromatographic techniques. The pH of the root and RET will also be measured using specific probes and microscopy.

Keywords: exDNA, Root, RET, xyloglucans, mannans, Glycine max, DNase I

**Intervenant*

TP N°354023

Studies of biological pathways involved in radiorasistant of cancer stem cells after hadron irradiation.

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Studies of biological pathways involved in radio resistant of cancer stem cells after hadron irradaiton Ionizing radiation (IR) kills cells primarily by generating reactive oxygen species (ROS) and cytotoxic double strand breaks (DSBs) in DNA. A certain fraction of cells in a cancer tumor is cancer stem cell. Cancer stem cells (CSC) have often-higher expression of antioxidant and more effective DNA repair systems that protect them from the effect of radiotherapy and lead to relapse consequently. The main objectives of my project are, to establish CSC line with combined deficiencies in oxidative stress response and DNA repair using genetic approaches such as CRISPR/Cas9 or shRNA, secondly to analyze the effect of combined antioxidant and DNA repair inhibitions with IR (x-rays, protons and carbon ions) on the radioresistant CSC. Finally, to find new pathway/s involved in radioresistance by proteomic and biostatistic analyses. The focus of research is to investigate how a cell or a patient deals with excess toxic levels of free radicals and elevated oxidative DNA base damage induced by exposure to hadron or any other type of radiation.

Keywords: Cancer stem cells, Nrf2, Hadron irradiation

Intervenant

TP N°352839

Cartilage engineering using vegetal biomaterials

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Cartilage is a connective tissue that maintains the tissue's shape (nose, ear), and absorbs shocks in the articulation. Those tissues are frequently damaged by traumatic, metabolic and/or congenital injuries. Therapeutic solutions remain limited and mainly target the articular cartilage due to the incidence of rheumatic diseases and their invalidating nature. Cartilage autograft provides an alternative for auricular cartilage repair. This approach, although the most successful, has many drawbacks such as surgical difficulties, donor site morbidity, infections, or necrosis. In that context, tissue engineering of auricular cartilage appears as an effective answer to reproduce cartilage with properties close to the native tissue for surgical treatment. Tissue engineering usually combines biomaterials with cells in an appropriate environment to develop *in vitro* tissues intending for grafting. For cartilage, chondrocytes or progenitor cells could be used. Because chondrocytes tend to dedifferentiate upon extension, progenitor from various origin (bone marrow, perichondrium, dental pulp...) are also widely used. An adequate biomaterial needs to enhance cell proliferation and differentiation while keeping a suitable shape. Indeed, natural or synthetic biomaterials frequently used in medicine tend to collapse. Therefore, natural decellularized biomaterials have been investigated. Decellularization consists in the elimination of cellular components, leaving only the extracellularmatrix that is recolonized with appropriate cells. Benefits are the absence of graft rejection and the opportunity of working on an already structured tissue. Based on this technique plant-based materials were tested for cell biocompatibility. The aim of my thesis project is to generalize the use of a vegetal scaffold in tissue engineering by seeding various human progenitor cells on a decellularized apple hypanthium to induce their chondrogenic differentiation. The neo-formed tissue will then be analyzed to determine the best cell type for in vitro chondrogenesis. I will evaluate its nature and structure, but also examine the expression of cartilage specific markers. Structural properties will be evaluated by biomechanical analysis to determine its elasticity, flexibility, and resistance. In the long term the idea is to validate this approach in tissue engineering and to decline it to obtain other tissues (muscle, bone...) as well as extending our work in vivo.

Keywords: tissue engineering, cartilage, decellularisation, vegetal scaffold

**Intervenant*

TP N° 354427

Functional interaction between Angiotensin II type 1 receptor (AT1) and Urotensin II receptor (UT)

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G protein-coupled receptors (GPCR) are involved in a broad range of physiological and pathophysiological processes. Some GPCRs are known to form functional homo- and/or heterodimers exhibiting pharmacological and functional features different from monomers. These heteromers constitute potential therapeutic targets, in particular in oncology. The vasoactive neuropeptides angiotensin II (AngII), urotensin II (UII), and UII-related peptide (URP) are the endogenous ligands of the GPCRs AT1 and UT, respectively. These peptides also exhibit pro-mitotic and pro-angiogenic properties and over-expression of AT1 and UT have been reported in various cancers, in particular in high grade glioma. The aim of this study was to investigate the existence of a functional interaction between AT1 and UT, putatively involved in gliomagenesis. By using a calcium mobilization assay in HEK-293 cells expressing tagged receptors (AT1-HA and UT-cMyc), we found that both tagged GPCRs conserved a nanomolar affinity for their endogenous ligands. Coexpression of AT1-HA/UT-cMyc receptors did not impact peptide potency and UII efficacy whereas AngII efficacy was greatly reduced (-40%). Incubation of cells with UII (10-11, 10-9, 10-7 M; 1h) provoked a dose-dependent decrease (-60% with 10-7 M UII) in AngII-induced calcium mobilization. Similar results were observed with a series of urotensinergic ligands including agonist and biaised ligands (URP, [Orn8] UII, and [Orn5] URP). In contrast, incubation of cells with AngII (10-11, 10-9, 10-7 M; 1h) did not modify UII activity. Binding studies led on HEK-293 cells coexpressing AT1-HA and UT-cMyc, revealed that UII (10-7 M, 1h) decreased both UT-cMyc and AT1-HA density, suggesting the existence of a functional interaction between UT and AT1, at least involving internalization. Immunocytochemistry and migration assays are in progress (on HEK-293 co-expressing tagged AT1/UT and human glioblastoma cell lines expressing both GPCRs) to characterize the existence of functional interactions between AT1 and UT linked to tumorigenesis processes.

Supported by: University of Rouen Normandie, Inserm, Gefluc.

Keywords: GPCR, angiotensin II, urotensin II, heterodimerization.

Intervenant

TP N°354490

NEMESIS: Interactions and chemical communication between toxic diatoms Pseudo-nitzschia and primary consumers (Copepods)

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Diatoms of the genus *Pseudo-nitzschia* are present in many marine ecosystems including the Bay of Seine. These micro algae produce a neurotoxin the domoic acid. They may be responsible for harmful algal blooms with human health and socio-economic consequences. Despite studies on shellfish and upper trophic level contamination (seabirds, marine mammals), the harmful effects of *Pseudo-nitzschia* on marine ecosystems are poorly understood. Very few is known about the impact of domoic acid in food webs, especially on mesozooplankton mostly represented by copepods. These organisms may operate as a vector toward higher trophic levels for the toxin. The presence of copepods has also been shown to modulate toxicity in some *Pseudo-nitzschia* species. The NEMESIS thesis and the INCIDENCE project are exploring for the first time in the Bay of Seine the interactions between *Pseudo-nitzschia* species and its planktonic primary consumers. Using controlled laboratory experiments the reciprocal influence of both partners will be studied, focusing on physiology and behaviour. The chemical communication involved in this interaction will be also explored (exo-metabolomic approach). Finally, an *in situ* approach will allow to validate laboratory observations. These studies will provide information on the influence of biotic factors on the toxicity and magnitude of *Pseudo-nitzschia* blooms in the Bay of Seine. Some biomarkers of exposure to domoic acid on Copepod will be also explored.

Keywords: Pseudo, nitzschia, Copepods, toxin

*Intervenant

TP N°354306

Steroidogenesis and androgen/estrogen signaling pathways in in vitro matured testicular tissues of prepubertal mice

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Childhood cancers represent 2550 new cases of pediatric cancers diagnosed each year in France. Cancer treatments such as chemotherapy have recognized toxicity on germline stem cells that could lead to infertility at adulthood. In order to preserve and restore the fertility of patients in remission, fragments of prepubertal testicular tissue will have to be frozen/thawed to be *in vitro* matured to produce spermatozoa that could be used in assisted reproductive technology. *In vitro* maturation has been developed in the mouse model to obtain spermatozoa from fresh or thawed prepubertal testicular tissues. However, despite the supplementation of culture media with retinol and vitamin E, *in vitro* sperm production remains a rare event. The production of estrogens and the signaling induced by these steroid hormones and androgen have never been studied in organotypic cultures. Since these hormones play an essential role in spermatogenesis and gamete quality, it appears necessary to ensure that their synthesis and mechanisms of action are not altered in *in vitro* cultured tissues. The aim of this project is therefore to study steroidogenesis and the androgen and estrogen signaling pathways during *in vitro* maturation of prepubertal mouse testicular tissues in order to increase the yield of spermatozoa obtained *in vitro*. The first results of this study show that the actors involved in the synthesis and mechanisms of action of steroid hormones have a decreased mRNA expression such as *Lhcgr* encoding the LH receptor, *P450scc*, *3 β-hsd,* and *Cyp19* encoding steroidogenic enzymes, *Esr1* encoding the estrogen receptor a and androgen/estrogen target genes (*Rhox5*, *Faah*). Furthermore, we observed Leydig cells expressing 3*β*-HSD after 30 days of organotypic culture but they seem to be partially mature with a weak expression of INSL3, a biomarker of Leydig cell functionality. Quantification of the percentage of mature Leydig cells and of the protein levels of the actors of androgen/estrogen signaling pathways will have to be performed.

Keywords: Steroidogenesis, Androgen, Estrogen, Testis, Mouse, Prepubertal, In vitro, Spermatogenesis

*Intervenant

TP N°354012

Impact of cancer and check-point inhibitors immunotherapies on emotional reactivity and cognitive functions in mice

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Cognitive dysfunction is an increasingly recognized complication of cancer and its treatments. Clinical studies make it difficult to discriminate the involvement of cancer, inflammatory/immune reactions, chemotherapy and targeted therapies, in the occurrence of cognitiveimpairments. Immunotherapies, such as immune checkpoint inhibitors, target the immune system to overcome cancer tolerance and to stimulate an antitumoral immune response. Among them, monoclonal antibodies targeting antigens expressed by activated T lymphocytes such as Lymphocyte-activation gene 3 (LAG-3) or programmed cell death protein-1 (PD-1), have emerged as the most encouraging approaches to treat cancer patients. Although anti-PD-1 agents have been shown to reduce high grade side effects compared with chemotherapy, neurological consequences such as migraine, encephalopathy and meningitis were recently described in cancer patients. We aim to understand potential plasma signatures, tumoral and cerebral mechanisms, vascular changes and neuroinflammatory responses underlying behavioral changes likely observed during or after cancer immunotherapies. Here we propose that the lymphatic network lining the dural sinuses and vascular structures called high endothelial venules, responsible for immune cells recruitment and infiltration of inflammatory cells into the central nervous system, could be favored in the context of cancer, in the absence or the presence of immunotherapy treatment. Before testing the direct impact of immunotherapies (anti-PD1, anti-PD-L1, or anti-Lag3) on behavioral functions, we first investigated the role of immune/inflammatory environnement built by cancer (B16F10-Ova or B16F10, less immunogenic tumors) in immunocompetent mice (C57B/l6, syngeneic model) or cancer cell lysate, on behaviors and brain functions. Our first data show that i) the presence of poor immunogenic melanoma cancer (subcutaneous xenografts) or its corresponding lysate (intradermal injection) was associated with subtle cognitive dysfunctions (novel object recognition test (NOR)) and that ii) more highly immunogenic melanoma (subcutaneous xenografts) and its respective lysate (intradermal injection) induced resignation behavior (Tail suspension test), anxiety (Elevated plus maze) and cognitive dysfunctions (NOR). To understand these observations, 2D and 3D immuno- histochemical analyses of mouse brains are currently under study while plasma and blood PBMC will be studied. The final objective of the work is to understand and potentially prevent future long-term consequences on survival and quality of life of cancer patients.

Intervenant

TP N°354504

Computerized intervention for the improvement of cognitive difficulties in women treated for breast cancer: implementation, follow-up and evaluation of the Cog-Stim research program

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Background: Cancer-related cognitive impairment is a common side effect of cancer treatment, with significant consequences for patients' quality of life, especially in elderly patients because of their frailty. Among non-pharmacological interventions, cognitive stimulation and physical activity are the most efficient for cognitive improvement. However,these interventions are difficult to implement in clinical practice. To overcome this limitation, computerized interventions have been developed, but feasibility and efficacy of these interventions still need to be investigated. We aim to evaluate the feasibility of using computerized interventions (combined or not) for the improvement of cognitive complaints reported by patients (adults and elderly) under treatment for breast cancer.

Method: the research program will involve multiple phases:

Pilot phase: Cog-stim study (ongoing, 18/20 included patients): Patients with cognitive complaints will participate to a 12 week online home-based program including two 20-minute sessions/week of cognitive stimulation (HappyNeuron), and two 30-minute sessions/week of supervised adapted physical activity (Mooven). The primary endpoint is the achievement of 70% of the program content. Patient satisfaction and the impact of the intervention on cognitive complaints, objective cognitive performance, as well as on anxiety, depression, sleep disorders and fatigue will be assessed. Cog-tabage study (ongoing 18/55 included patients): patients undergoing treatment for breast cancer, aged 70 years and older will be asked to perform three 20-minute sessions of cognitive stimulation on a tablet (HappyNeuron). The first session is for familiarizing with the software under the supervision of a neuropsychologist, while during the other two sessions patients will use the software unsupervised. The main criterion is the completion of atleast three exercises proposed in 10 minutes during one of the two autonomous sessions. Patients' satisfaction will also be investigated.

Randomized phase: Cog-stim2 study: longitudinal (follow-up at 3- and 9-months post-intervention) and multi-site study to evaluate the benefit of computerized cognitive stimulation. The study will be planned upon feasibility studies' results.

Discussion: the Cog-stim research program aims at identified the best computerized intervention for cancer-related cognitive impairment. This will allow us to better support patients in their rehabilitation and improve their quality of life, especially during the current healthcrisis.

Keywords: breast cancer, cancer, related cognitive impairment, computerized, intervention, cognitive intervention, cognition

Intervenant

Characterization of the ionomic signatures of plantssubmitted to nutritional deficiencies and some potentially involved mechanism

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Characterization of the ionomic signatures of plants submitted to nutritional deficiencies and some potentially involved mechanism It is suggested the ionome composition (i.e. macro, micro and beneficial elements) of plant tissues, resulting from multiple interactions between nutrients, can reveal plant physiological status. This study aims to identify elemental interactions and hence ionomic signatures, resulting from different mineral deficiencies in Brassica napus and Triticum aestivum. Therefore, plants were submitted to 17 individual nutritional deficiencies (N, Mg, P, S, K, Ca, B,Cl, Mn, Fe, Ni, Cu, Zn, Mo, Na, Co and Se) and harvested before reduction of growth. The main objectives were to i) analyze plant tissue ionomic signatures and compare the response of the both species, ii) identify some subjacent metabolic pathways, iii) and quantify the net remobilization of each nutrients. In both species, the main results revealed elemental interactions already described that could be the consequence of the up-regulation of nonspecific root transporters, hence increasing the uptake of other elements available in nutrient solutions. For examples, a strong increase of divalent cation uptake was found under Fe deficiency, as it was also the case for Mo under S deficiency. An original interaction concerned a stimulation of vanadium uptake in plants submitted to S deficiency, probably a result of an over-expression of root sulfate transporters confirmed notably by ionome analysis of Sultr1;1 and Sultr1;2 knock-out Arabidopsis lines. Our study showed also another original (but negative) interaction between N and Na that was also supported using Arabidopsis lines knock-out for genes encoding nitrate transporters. Finally, in rapeseed roots deficient in macronutrients, specific and mutual physiological andmolecular processes were identified using transcriptomic and metabolomic approaches.

Keywords: Ionome, Nutrient deficiencies, Nutrient interactions, Oilseed rape, Wheat

*Intervenant

P N°352215

POSTER

Chemoattraction of glioma cells in a local hydrogel trap and immune control associated with improved survival and cognitive functions in a mouse model of glioblastoma resection

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Glioblastoma (GB) is the most aggressive primary tumor of the central nervous system and represents the third cause of death in adult cancer patients. The prognosis remains poor mainly due to the invasiveness of glioma cells linked with the radio and/or chemoresistance, the presence of a blood-brain barrier and the GB-induced immunosuppressive environment warranting the development of new therapeutic strategies. Here, we propose to use a local delivery system based on a biocompatible hydrogel containing the chemopeptide urotensin II (UII) or a biased synthetic analog [Pen5, D-Trp7, Dab8]-UII (4-11) (DAB8), to "trap" GB cells and/or to control immune cells leading to tumor regression and neurological benefit, in a mouse model of GB growth after resection. In vitro, invasive capacity of human U87 and 42MG or murine GL261 and CT2A GB cells was stimulated toward a UII gradient concentration loaded into hyaluronic acid supplemented with collagen, adipic acid dihydrazide, jeffamine or polyoxazolines hydrogels, PNIPAAm- PEG hydrogels or thrombin-fibrin glue, tested in cloning ring and Boyden chamber assays.In vivo, injection of UII- or DAB8-loaded thrombin-fibrin glue into the cavity after resection of intrastriatal murine GL261 and CT2A GB in C57BL/6 mice significantly improved mouse survival compared with tumor and resected experimental conditions. Neurological statuswas also tested before and after GB resection. We found that GL261 and CT2A cell-bearing mice expressed altered spontaneous activity, emotion and cognitive functions. Intracavity injection of the glue improved resignation and anxiety and increased motor activity and cognition with a best cognitive recovery with UII and DAB-8-loaded glue groups. Ex vivo brain analyses revealed high expression of UT and UII in some GFP-positive cells and macrophageswithin GB core and at the interface with the normal brain, while GB cells expressing UT were migrating along tortuous podocalyxin-positive vascular components. In brains bearing hydrogel + UII glue, GFAP-positive astrocytes and F4/80-positive macrophages were significantly highly recruited in the border of the cavity, compared with the other conditions.
There data suggest that a local glue containing UII may trap GB cells and remodel the tumor microenvironment responsible for survival and cognitive improvements, providing new therapeutic options in the treatment of GB.

Keywords: Glioblastoma, Resection, UII, Hydrogel, Invasion

Intervenant

P N°354473

Emotional responses to positive and negative feedingexperience in the common cuttlefish (Sepia officinalis)

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Since 2013, cephalopod molluscs have been included in the Directive 2010/63/EU on the protection of animals used for scientific purposes. Paradoxically, there is no ethological method to evaluate their welfare in laboratory conditions. Cuttlefish brain/body ratio is comparable to that of many vertebrates. Their skin is covered with innumerable pigmented and contractile cells controlled by the nervous system, they allow the cuttlefish to instantaneously change of colour and body patterns. Body patterning is known to be involved in camouflage and in inter- and intra-specific communication. In the present work we hypothesise that they are also involved in emotional responses (project ANR ETHiCs 18-CE02-0022). In nine juveniles of cuttlefish (*Sepia officinalis*), body pattern changing were measured during prey hunting, in a positive context (free shrimp, preferred prey) and negative context (shrimp in a glass tube, unreachable preferred prey). Our results show that 1) seven body patterns, out of tens, may be considered as emotional response 2) body pattern changes during hunting are more intense in negative than in positive contexts. Based on these observations, we proposed the use changes of body patterns to assess emotional states and welfare in *S. officinalis*.

Keywords: Predation, Welfare, Emotion, Cuttlefish, Body patterns

*Intervenant

P N°353294

Endothelial deficiency in Pkd1 impairs the development of arterio-venous fistula

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INTRODUCTION:

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. It is mainly due to mutations in PKD1 which encode for polycystin 1 (PC1). PC1 is a transmembrane protein, located on the primary cilium of endothelial cells and epithelial renal cells, and acts as a blood or urinary flow sensor. In ADPKD, this mechanotransductory mechanism is altered, and leads to cyst formation and vascular abnormalities. In ADPKD, after decades, patients may reach End Stage Renal Disease (ESRD) requiring Hemodialysis. An arterio-venous fistula (AVF) created by an anastomosis between an artery and a vein is the preferred vascular access to perform hemodialysis. However, in ADPKD there are more immediate AVF failures, the mechanisms of which remain unknown.

RESULTS:

We developed a model of aorto-cava AVF in C57bl/6J mice. Two groups were compared: mice with endothelial specific knock-out of Pkd1 (iCdh5-cre; Pkd1del/del) induced at birth vs control mice (Pkd1lox/lox). We performed doppler ultrasonography at D0, D1 and D14 after surgery. The overall survival did not differ between groups (82.3% in iCdh5-cre; Pkd1del/del vs 81.3% in Pkd1lox/lox). An increase in venous flow demonstrated success of AVF in both iCdh5-cre; Pkd1del/del (D0:10.3µL/s vs. D1:32.4µL/s p=0.02) and Pkd1lox/lox mice (D0:11.3µL/s vs. D1:30.0µL/s p=0.07). In iCdh5-cre; Pkd1del/del mice the venous blood flow did not increase significantly between D0 and D14 (D0:10.3 vs. D14:29.6µL/s p:ns), as opposed to control mice (D0:11.3 vs. 49.0µL/s p< 0.01), demonstrating maturation failure in iCdh5-cre;Pkd1del/del mice.

CONCLUSION:

The results of this study demonstrate that endothelial Pkd1 is required for the development of AVF.

Keywords: Autosomal dominant polycystic kidney disease, polycystin 1, arterio, venous fistula

Intervenant

P N°353985

POSTER

Evaluation of plasticizers from protective gloves: determination of the content of plasticizers in vinyl gloves and in vitro toxicity studies on microvascular endothelial cells

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Over the past few years, exposure to plasticizers have been linked with multiple effects on human health, including endocrine disruption and cardiovascular diseases. Plasticizers are wide use additives which are incorporated in many products including protective gloves. The frequent use of gloves is a source of dermal exposure to plasticizers because it favours the occlusion of skin barrier. It can contribute to their transcutaneous migration since they are not covalently bound. Thus, they may affect dermal microvascular endothelial cells. Despite the large use of gloves, their plasticizers content have been poorly studied. This study aims to investigate the toxicity of plasticizers found in gloves on microvascular endothelial cells by evaluating their effects on mitochondrial activity. The plasticizers content of five vinyl gloves was analyzed using a GC-MS method previously developed in our laboratory for the simultaneous determination of 11 plasticizers. The effects of the main plasticizers found in gloves were evaluated on a human dermal microvascular endothelial cell line (HMEC-1) after single exposures on four consecutive days. Plasticizers were tested at a concentration of 15 mg/L individually and in the mixture. Their effects on mitochondrial metabolic activity was measured by using MTT assay and the cellular levels of adenosine nucleotides (ATP, ADP and AMP) was measured by HPLC-DAD. The analysis of the vinyl gloves revealed three main plasticizers, namely di-(2-ethylhexyl) phthalate (DEHP), di-(2-ethylhexyl) terephthalate (DEHT) and di-isononyl phthalate (DINP). Amounts were reported up to 44.44% (w/w). Cell exposures to the found plasticizers showed alterations on the mitochondrial activity. For all conditions, an increased cell viability was observed, it was then followed by a decrease excepted for DEHT. First results on levels of adenosine nucleotides showed modifications after three hours of exposure with all three plasticizers individually but not in combination. Further experiments are in progress to complete these results. This study throw light on plasticizers in vinyl gloves with reported amounts that raise concerns regarding health. In vitro studies provide new knowledge on the endothelial toxicity of individual and mixture effects of DEHP, DINP and DEHT at the microvascular level. These findings contribute to fill knowledge gaps on the subject.

Keywords: Plasticizers, vinyl gloves analysis, microvascular endothelial cells, cytotoxicity, mitochondrial activity

*Intervenant

P N°354342

Impact of targeted irradiation on the healthy brain tissue and cognition: longitudinal and multiparametric study in the rat

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Background: Although radiation therapy improves the patient prognosis with brain cancer, it also affects the healthy brain tissue, a phenomenon recognized to induce irreversible cognitive deficits in the long-surviving patients. Sparing the surrounding healthy tissue and organs at risk is nowadays more used clinically, but the interest of new modalities in radiation therapy to reduce brain radiotoxicity is fragmentary at preclinical level. Animal models are of major importance to elucidate the effects of targeted radiation on the brain. Thus, this study aims to better understand the effects of targeted brain irradiation on the tissue integrity and cognition in a rat model. **Methods:** Adult male Wistar rats were divided into control (CTL) and irradiated (IR) groups. Fractionated irradiation (total dose of 30Gy) was applied on the right hemisphere using a preclinical irradiator (X-RAD 225Cx, Cyceron). A battery of behavioral tests was performed longitudinally during 6 months to analyze short-term (novel object recognition, NOR) and long-term (passive avoidance, PA) memories as well as spatial learning and reference memory (Morris water maze, MWM). Locomotor activity and anxiety-like behavior were assessed by actimetry and elevated plus maze (EPM) respectively. Sequential MRI (T2) analyses were also performed. **Results:** Long-term memory was not altered in IR group unlike short-term memory evidenced by the decrease in the recognition index (NOR) at day 3 post-irradiation compared to CTL group. The parameters assessed during the training phase of the MWM test showed a significant alteration of learning in the IR rats at 2 weeks and 5 months after irradiation relative to CTL rats. Actimetry revealed no major difference in locomotor activity between the groups, whereas exploration activity of IR rats was significantly higher at 2 weeks, 2 months and significantly lower at 4 months following irradiation. IR rats showed a significant anxiety-like behavior at 2 weeks and 5 months (EPM). MRI did not show any radio-necrosis nor edema after irradiation but a significant reduction of the irradiated hemi- sphere volume was observed. **Conclusion:** The data show that targeted brain irradiation induces significant brain damage and cognitive deficits in rat. This animal model could be used to test therapeutical strategies.

Keybords: Radiotherapy, Brain, MRI, Physiopathology, Cognitive decline

**Intervenant*

P N°354230

SEX-AND TIME-DEPENDENT PREVENTIVE EFFECTS OF MAGNESIUM SULFATE AND A BUTYRATE IN A NEONATAL MOUSE MODEL OF HYPOXIC-ISCHEMIC INSULT

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Neonatal brain lesions can induce cerebral palsy (CP) defined as permanent disorders of movements and posture. Preterm birth and hypoxic-ischemic (HI) events are risk factors of CP. Moreover, boys display a greater vulnerability to develop CP than girls do. A meta-analysis showed that magnesium sulfate (MgSO4) administration to mothers at risk of preterm delivery reduces the risk of CP by 32%. Within the laboratory, in a mice modelof perinatal HI, generating lesions similar to those found in preterms with CP, MgSO4 was shown to prevent the HI-induced inflammation and sensorimotor deficits at short term. However, at long term, MgSO4only presented a partial effect. We designed a new project to try to prolong MgSO4 beneficial effects by associating it to a second molecule presenting neuroprotective properties. A butyrate caught our attention because it presents these properties. Moreover, in pathological conditions, this molecule modulates DNA acetylation, resulting in a modulation of genic expression. The main goals are: to characterize, at short and long terms, the effects of MgSO4 alone or administered with butyrate, on white matter injuryand on motor and cognitive disorders induced by the neonatal HI; to investigate if theseeffects depend on sex; to search for potential deleterious proper effects of the drugs.

To address these questions, postnatal day 5 mice underwent an unilateral carotid ligature followed by hypoxia, with MgSO4 and butyrate injected alone or co-administered. This latter was named "cocktail" to simplify the reading. At short term, we evaluated MgSO4 and cocktail efficacies on the sensorimotor abilities and on the cerebral tissue integrity. At long-term, we investigated white matter integrity and measured behavioural skills. In non-HI pups, we searched for potential deleterious proper effects of the treatments.

At short term, the cocktail prevented HI-induced histological and behavioural alterations, as did MgSO4 alone. Indeed, the cocktail did not alter short term MgSO4 preventive effects. However, contrary to MgSO4, the cocktail succeeded in preventing HI-induced motor and cognitive alterations at long term. The next step will be to decipher butyrate mechanisms of action by focusing on DNA acetylation levels, remyelination and inflammation.

Keywords: Prematurity, Cerebral palsy, Hypoxia, ischemia, MgSO4, Butyrate

Intervenant

P N°354125

High temperature patterns determine seed yield and quality in oilseed rape in the context of climate change

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High temperatures during the crop reproductive stage impact seed yield and quality. The changing climate will require consideration of the effects of high temperature events that differ from their intensity, their duration and their frequency over the seed quality-building stages. The impact of these features deserve to be investigated at the light of induced thermo-sensitization which can lead to alleviate expected negative impacts. In our work, maturing seeds of the sulphur-demanding crop, oilseed rape, were exposed to several temperature sequences that varied in intensity, duration and frequency at the onset of seed maturation. Results-measured in seeds that were at the onset of maturation when the temperature stress occurred-indicated that (i) the longer the cumulated duration of the temperature stress, the more negatively impacted the quality criteria with decreased fatty acids (FAs) concentration, increased $\omega 6$: $\omega 3$ ratio, lower seed membrane integrity and increased seed dormancy and (ii) a mild stress event prior to heat peaks had an alleviating effect on the negative impact of the later heat peaks (priming effect) on seed nitrogen, desiccation tolerance and the phytohormones involved in thermoinhibition. Sulphur restriction was positive on FAs, protein concentration and negative on breaking dormancy. In addition, sulphur supply interfered with temperature modality, features such that positive impact of sulphur limitation on boosting oxidative response were cancelled with intense late heat peaks. This work provides insights to define thermopriming protocols in relation to the timing of quality building processes, their respective optimal temperature and adequate sulphur supply.

Keywords: agriculture, climate change, thermostress, oilseed rape, sulphur

*Intervenant

P N°353315

Molecular characterization of the Root Extracellular Trap (RET) in ryegrass (Lolium perenne), a fructan-producing plant

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The root cap of many plant species releases atypical living cells called “Root Border Cells” or “associated root cap derived cells (AC-CDs) embedded in a surrounding mucilage (Driouich *et al.*, 2019). This mucilage, enriched in cell wall glycomolecules such as pectins and arabinogalactan-proteins (AGPs), acts in conjunction with the AC-DCs to form a structure defined as Root Extracellular Trap (RET) by analogy with the Neutrophil Extracellular Trap (NET) involved in mammalian immunity (Driouich *et al.*, 2013). The RET was shown to play a key role in root microbe-interactions (Hawes *et al.*, 2000; Cannesan *et al.*, 2012) but its precise composition and function need to be more clearly defined. Fructans (polymers of fructose synthesized by 15 % of plant species) are believed to be involved in the ‘Sweet Immunity’ (Bolouri-Moghaddam and Van den Ende, 2013). Our hypothesis is that fructans could be involved in root defense and be part of the RET of the fructan-producing plant. In this study, we used ryegrass (*Lolium perenne*), as a fructan-producing plant model to characterize the molecular composition of the RET using immunocytochemistry, anti-cell walls glycan antibodies, and confocal microscopy. Our immunocytochemical data showed that mucilage from ryegrass root was enriched in AGPs epitopes. However, there are no antibodies available directed against fructan epitopes. In order to investigate fructans localization within the root system, we solicited BIOTEM company to design and produce monoclonal antibodies. Two novel monoclonal antibodies (mAbs), named 15A6 and 9H2, were selected as promising to bind to fructans. Here, we provide their characterization using dot blot analysis and a wide range of standards including different oligosaccharides and polysaccharides. Immunolocalisation was also performed at the confocal microscopy level on the root of ryegrass. Overall this study will give us novel insights into the function of fructans in fructan accumulating species that might lead to the development of innovative crop protection strategies.

Keywords: Border cells, mucilage, Root extracellular trap, arabinogalactan, proteins, fructans, plant immunity, ryegrass.

**Intervenant*

P N°354340

Deciphering the role of miRNA in the control of catecholamines secretion by pheochromocytomas

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Pheochromocytomas (PCC) are rare neuroendocrine tumors of the adrenal medulla gland that secretes high levels of catecholamines into the bloodstream. This leads to serious clini- cal repercussions such as hypertension. The causes of this secretory dysfunction are poorly understood, however, several studies suggest that miRNA, post-transcriptional inhibitors, may play a role in controlling this regulated secretion pathway (RSP). We employed bioinformatics and cellular strategies to identify and characterize the involvement of miRNA in catecholamines hypersecretion of PCC. Thus, using the STRING-DB webtool, an enrich- ment analysis of gene sets associated with exocytosis, the final step of RSP, allowed us to identify 96 genes essential for RSP. We used two bioinformatic miRNA target prediction tools, mirDIP and miRabel, which allowed us to identify 221 highly conserved miRNA. We also exploited TCGA transcriptomic data, via the oncomiR webtool, to further select the 186 miRNA that were inversely co-expressed with their potential targets. Finally, the use of gene expression data (GSE19422) and miRNA (GSE29742) from PCC and normal adrenal medulla samples allowed us to highlight an interaction sub-network in which the 28 miRNA and 38 genes involved in exocytosis are deregulated in PCC. In order to study the effect of these miRNA on the activity of RSP, we developed a secretion test based on a luminescent reporter. For this purpose, the human growth hormone (hGH1) gene, which is specifically expressed in the dense-core secretion granules of the RSP, was fused with the Nano-luciferase (Nluc) and then expressed in a rat PCC cell line (PC12-2luc). In a preliminary assay with miR-34a-5p, one of the 28 identified miRNA, we observe an accumulation (+67%) of the luminescent reporter in PC12-2luc cells under basal conditions, compared to the negative control. Moreover, under stimulated conditions (BaCl2), we observe a decrease (-39%) in Nluc secretion, confirming the repression of miR-34a-5p on the RSP. Since our approach is validated, we are currently studying all the 28 identified miRNA. The characterization of miRNA affecting RSP, including miR-34a-5p, and their interaction network with the 38 identified genes, will allow to better understand the deregulation of secretion mechanisms in neuroendocrine tumors such as PCC.

Keywords: microRNA, pheochromocytoma, regulated secretion pathway

Intervenant

POSTER

Viability assay of A549 and HaCaT cells after direct exposure to spores and ethyl acetate extracts of Aspergilli of the Versicolores section

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Indoor air quality and exposure to fungal bioaerosols is a major concern in Europe where people spend 80-90% of their time indoors. According to the WHO, 30 to 50% of european homes have moisture problems that ease the development of moulds with major health consequences. Aspergilli of the Versicolores section are recurrently found in the air of these degraded habitats. Phylogenetic studies have identified new species in this taxon, leading us to characterise fungal isolates belonging to this section and to evaluate their cytotoxic effects on the A549 and HaCaT cell lines.

Spores suspensions and ethyl acetate extracts were obtained from colonies of Aspergillus amoenus, A. creber, A. jensenii, A. protuberus and A. sydowii grown for 21 days on MEA medium at 25°C. For each concentration of spores (101/102/103/104/105 spores/mL) or extract (25/50/125/250 µg/mL) tested, 5 replicates were performed. After 72 hours of exposure, cell were stained with sulforhodamine B and the optical densities were measured by UV-visible spectrophotometry.

After 72 hours of exposure, no spore suspension caused a decrease in cell viability. Extracts of A. creber and A. jensenii caused a significant decrease in viability in A549 cells (IC50: 116.91 and 148.85µg/mL respectively). All extracts except A. sydowii caused a significant decrease in viability in HaCaT cells with an IC50 of less than 25µg/mL for A. creber, 69.84µg/mL for A. amoenus, 209.26µg/mL for A. jensenii and 229.97µg/mL for A. protuberus.

Direct exposure of cells of the A549 and HaCaT cell lines for up to 72 hours to fungal spores of the isolates of the five Aspergillus species studied did not show toxicity at the concentrations tested corresponding to those found in the bioaerosols of degraded habitats. However, ethyl acetate extracts from these same species and under the same exposure conditions showed a decrease in survival in these cell lines.

Keywords: Bioaérosols, Aspergillus, Versicolores, Viabilité cellulaire, A549, HaCaT

*Intervenant

P N° 354193

Ex vivo and in vivo approaches to study the dynamics of glioma cell invasion within the white matter

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Glioblastoma is a glioma, a brain tumor of glial origin. It is the most common and aggressive primary tumor of the central nervous system in adults with a median survival rate of 14,6 months after standard treatment. This poor prognosis of this cancer is related to the invasiveness of glial tumor cells that infiltrate the brain parenchyma mainly along blood vessels and in white matter boundaries, leading to a high propensity for tumor recurrence. While migration along the blood vessels is well characterized, the mechanisms relaying tumor invasion within the white matter are still poorly understood. White matter, which is mainly composed of myelinated and unmyelinated axons, sustains neuronal influx propagation over large distances. Whether those electrical activities influence glioma cell invasion is not known. To study functional impact of neuronal activity on glioma cells invasion, optogenetic experiments are planned. As a first step, *in vivo* and *ex vivo* experimental approaches have been designed to evaluate the dynamics over time of glioma cell invasion. Infiltration of glioma cells in the tissue was detected and quantified by immunostainings and subsequent imaging of free-floating brain slices and 3D whole brain after clearing. The first approach consisted in the stereotaxic injection of murine and human glioma cells (Green Fluorescent Protein GFP+) within the corpus callosum of immunocompetent C57Bl6/J and immunodeficient Nude adult mice. According to cell lines, long-distance infiltrations within white matter tracts are observed. In addition, an *ex vivo* model has been developed: organotypic brain slice cultures from C57Bl6/J mice were grafted with murine and human glioma cells, allowing tumoral progression monitoring over weeks. Theses two experimental approaches will be ultimately used to study the impact on neuronal activity on tumoral cell migration along vessels and axons using pharmacological approaches (TTX, High K+) and optogenetics. This study will unravel the functional impact of the microenvironment activity in glioma progression, within the white matter.

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Keywords: Glioblastoma, White matter, Axons, Vessels, Migration

**Intervenant*

P N°354472

POSTER

Interest of HDAC inibitor belinostat, in monotherapy or in association with Bcl-xL or Mcl-1 inhibitors, in ovarian cancer treatment

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Context and objective: Identification of innovative therapeutics strategies represents a major issue to improve the treatment of ovarian cancer, first cause of death by gynecological cancer. Our Unit showed that ovarian cancer cell apoptosis seemed to be induce by the inhibition of anti-apoptotic proteins Bcl-xL and Mcl-1 and/or by promoting the expression of their parters pro-apototic Bim and Puma. Our objective was to determine if the belinostat, an HDAC inhibitor currently use in USA for the periferal T lymphoma treatment, could modulated the genetic expression of these differents targets and be a effective therapeutic strategy in ovarian cancer. **Methods:** Treatment efficacity was already evaluated in vitro in ovarian cancer cell lines SKOV3 and IGROV1-R10, by various technics uncovering apoptosis. Then, these results were validated by an *ex-vivo* study in tumor organoids establish in our lab from patients' tumor. Response to treatments was studied by morphologic and viability organoids analysis. **Results:** In our ovarian cancer cell lines, belinostat has a cytostatic effect and, at higher concentration, a cytotoxic effect related to apoptosis induction. On the other hand, it increases considerably Bim and Puma proteic expression and inhibits partially Bcl-xL proteic expression. At cytostatic concentration, belinostat associated with BH3-mimetics molecules inhibiting Bcl-xL (ABT-737) or Mcl-1 (AMG 176) induces effectively apoptosis in ovarian cancer cell lines. The study carried in tumor organoids, models more representative of patients' tumor, confirmed the belinostat efficacity in monotherapy or combined with ABT-737 or AMG 176.**Conclusion:** All the data suggest the interest of therapeutics strategies including belinostatin ovarian cancer.

Keywords: Ovarian cancer, Belinostat, Apoptosis, Bcl, 2 family proteins, Organoids.

Intervenant

P N°354458

Legumes carbon and nitrogen rhizodeposition: study of their variability and influence on soil enzymes activities

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Soil constitutes one of the richest ecosystems on earth and harbors essential organisms for a wide range of processes [1]. Enzymes, mainly originating from microorganisms, are a direct expression of metabolic needs in the soil [2]. By their presence, plants influence soil processes and legumes are particularly effective. They improve soil quality and provide several ecosystem services [3]. Their specific use of atmospheric N2 constitutes a source of entry of N into the soil. This involves rhizodeposition, a process of the release of different compounds from the roots into the soil, including tissue senescence [4]. Through this process, legumes contribute to C sequestration, enrich the soil with N and stimulate soil microorganisms [5]. This study aims to determine the variability of C, N rhizodeposition with different legumes plants and the influence on enzymatic activities related to the N, C, P cycles in the soil. Four legumes (pea, faba bean, white clover and Italian Clover), Wheat was used as non-fixing control plant. The N and C fluxes in the plant-soil system are studied using isotopic labeling of the culture substrate with mineral N enriched in 15N (quantification by 15N dilution) and atmospheric labeling 13CO2 (monitoring of 13C between plants and soil). Enzymatic activities related to the C, N cycles: B-Glucosidase (BGLU); Arylamidase (ARYLN); N-acetyl-glucosaminidase (NAG) and the P cycle: Phosphatases (PHO); Alkaline and Acid Phosphatases (PAL; PAC) are measured in soil at 2 stages (vegetative and reproductive). The results demonstrate a variation in the enzymatic profile of the soil depending on the species and plant development stage. The presence of faba bean and clovers presented higher level of the enzymatic activities in soil compared to pea. The differences between species are related to the amount of C and N rhizodeposited. We have also observed that the species having rhizodeposited more C (here the Italian clover) is not the one which rhizodeposited the most N (here the faba bean). Other analyzes are underway in order to understand physiological factor that explains differences in C and N rhizodéposition. We will also analyze the link between these differences in rhizodeposition and soil microbial communities.

Keywords: Soil, legumes, microorganisms, enzymatic activities, rhizodeposition

Intervenant

P N°354461

POSTER

Role of the endoplasmic reticulum-resident selenoprotein T in POMC neurons from the arcuate nucleus

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Selenoprotein T (SELENOT, SelT) is a vital selenoprotein in mammals and is the most conserved member of this family in Eukaryotes. It is present in the endoplasmic reticulum (ER), interacting with the oligosaccharyltransferase complex (OST), where it maintains protein homeostasis. Thus, SELENOT expression increases with metabolic demand and its expression is regulated by AMPK. Ins-Cre-SELENOT KO mice show decreased insulin secretion and impaired glucose tolerance. In addition, high levels of SELENOT transcript aredetected in POMC neurons of the arcuate nucleus, which are among the first exposed to peripheral signals. Furthermore, SELENOT expression increases after exposure to stresses and exerts a neuroprotective effect. Because oxidative and ER stress are found in POMC neuronsin patients with obesity, associated with low plasma levels of α-MSH, a POMC-derived peptide, and decreased leptin and insulin sensitivity in a mouse model of diet-induced obesity,we suggested that SELENOT may have a neuroprotective role in POMC neurons exposedto physiological anorexic signals and/or to a lipotoxic pathophysiological stress. Using a mouse hypothalamus-derived POMC cell line, we found as expected that SELENOT expression is increased twice in response to anorexic signals (insulin or leptin stimulation) or after lipotoxic stress (palmitate treatment). Depletion of SELENOT by CRISPR-Cas9 leads to an increase in the size of the ER and of the cells, which appear more flattened. Immunocytochemistry coupled to confocal imaging revealed cell trafficking and cell polarity disorganization. Specifically, the prohormone POMC remained retained in the ER in KO cells. An increase in the level of KCP2, a subunit of OST, was found, and PNGase F treatment combined with mass spectrometry analysis showed specific alteration of N-glycans despite an overall normal N-glycome profile. Using concanavalin A to label N-glycans, we observed their nearly complete loss at the membrane level. Altogether these results support a role for SELENOT in the secretory pathway, proteostasis and neuroprotection of POMC neurons, for which inflammation and ER stress lead to redox status alteration and hypothalamic nutrient sensing impairment linked with POMC misfolding. Further studies are ongoing to understand the function of SELENOT in these neurons,and its effect in food intake regulation.

Keyword: Selenoproteine T, Neuroscience, Endoplasmic Reticulum, Glycosylation

*Intervenant

P N°354435

The biological effects of long-term repeated exposure of monocyte-derived macrophages cells (MDM) and human bronchial epithelial cells (NHBE) to indoor airborne particles in vitro

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The biological effects of long-term repeated exposure of monocyte-derived macrophages cells (MDM) and human bronchial epithelial cells (NHBE) to indoor airborne particles in vitro. Exposure to indoor air pollution is associated with adverse health effect. In this study, an experimental strategy using monocyte-derived macrophages cells (MDM) and primary culture of human bronchial epithelial cells (NHBE) grown at an air–liquid interface was developed to investigate the long-term effects of repeated exposure to a standard reference material of airborne particulate matters SRM 2585 (Organic contaminants in house dust), separated into PM2.5 and PM10 respectively, and indoor dust samples collected from offices (working space) by our specialist. The experimental strategy we developed plays a key role in cell proliferation and cell differentiation. It is suitable for investigating in greater depth the long-term effects of particles on bronchial epithelial cells repeatedly exposed to indoor air contaminants in vitro. As a first step, the capacity of PM to induce cytotoxicity, oxidative stress, genotoxicity and inflammatory response, in both cell models, is evaluated by different tests. Moreover, the fate of the particles and epithelial differentiation are studied after the treatment. As a second step, the possible interaction between the two cell types in term of inflammatory response will be assessed after a co-exposure of treated NHBE to basal medium harvest from previously treated MDM.

Keywords: NHBE, MDM, PM, indoor dust, SRM 2585, cytotoxicity, oxidative stress, genotoxicity, inflammatory response, interaction and co-exposure

**Intervenant*

P N°352427

Effects of chronic exposure to metals from the dissolution of a galvanic aluminium anode in the Pacific oyster Crassostrea gigas

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Human activities contribue to the discharge of an increasing number of chemical substances into the environment and particularly into aquatic habitats. The increasing installation of anthropogenic structures in sea (ships, port installations, wind farms, oil platforms) implies the implementation of corrosion protection systems and it is common to use galvanic anodes. This type of protection consists of controlling the corrosion of a metal surface by means of an anode made of a metal whose electrochemical potential is lower than that of the metal to be protected. The galvanic anode then undergoes oxidation and releases various metals in the form of ions or oxyhydroxides into the marine environment. The main elements of galvanic anodes are aluminium, zinc or magnesium. Very few studies have investigated the fate of metals released from galvanic anodes and their potential effects on marine organisms. The main objective of our study is to investigate the chronic toxicity of galvanic aluminium anodes on the Pacific oyster, *Crassostrea gigas*, a species widely used in ecotoxicological studies. Oysters were exposed for three months to three concentrations of aluminium (50, 100 and 300 μg/L) using an experimental device simulating the dissolution of a galvanic anode. After 24 hours, 1 week, 1 month, 2 months and 3 months of exposure, we studied a battery biomarkers such as immune parameters (phagocytic activity, stability of lysosomal membranes, reactive oxygen species), analysis of the progress of gametogenesis, the metabolic state of the oysters with the quantification of malondialdehyde as well as glycogen energy reserves. In addition to these biomarkers, the bioaccumulation of the different constituent metals of the anode was measured in oysters at different exposure times. The majority of the biomarkers tested did not show any modulation even at the highest concentration except for phagocytic efficiency, MDA content in the digestive gland and glycogen reserves which decreased after 3 months of exposure. But for environmentally relevant concentrations, no significant changes in biomarkers were observed.

Keywords: galvanic anode, oyster, aluminium, marine pollution, ecotoxicology, biomarkers

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Exploration of the impact of the elevation of two proteins identified by a proteomic approach in a human podocyte model of Fabry disease.

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Fabry disease is a recessive X-linked lysosomal disease (LD) due to a deficit in alpha-galactosidase A, an enzyme involved in the glycosphingolipid metabolism. Regarding therapeutic strategies, two enzyme replacement therapies are currently available, as well as a pharmacological chaperon. If they significantly improve the course of the disease, they do not completely stop the course of the disease. Plasma globotriosylsphingosine (LysoGb3) a derivative of Gb3, the storage product, is used as a biomarker for disease diagnosis and monitoring. However, false negatives in very-late-onset forms and in some female patients have been reported, and LysoGb3 is not a robust marker of treatment response. Further understanding of the molecular pathophysiology is needed to improve management. Tebani et al. (J Clin Med 2020) applied a targeted proteomic approach and assessed 40 proteins in 69 plasma samples from the French Fabry cohort as well as 83 samples from healthy subjects and samples from patients with other LDs. The study yielded a clear elevation of fibroblast growth factor 2 (FGF2) and of interleukin 7 (IL-7) was observed in Fabry patients compared to other samples. We aimed explore the implications and impact of the elevation of these two proteins in Fabry disease. A human immortalized podocyte model of Fabry disease generated using CRISPR/Cas9 technology will be used as well as control podocytes provided by the same laboratory will be used. Different pre-treatments and treatments will be applied before measurement of Gb3 concentrations and α-galactosidase A enzyme activity. The cell cultures will be pre-treated with recombinant human FGF2, recombinant human IL-7, or both, and then treated with a recombinant α-galactosidase A enzyme. Gb3 and enzyme activity will also be measured in control conditions. Proteomic approaches can unveil new mechanisms and pathways. Their exploration will allow to maximize the potential of these new technologies.

Keywords: Fabry disease, FGF2, IL7, biomarker, podocytes, proteomics

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