Belgian Society for Cell and Developmental Biology
Spring meeting, June 13th, 2019

Experimental models for human diseases

Deadline for abstract submission : May 30th, 2019
Venue: Château de Colonster, Liège
Information: www.bscdb.be

ABSTRACT BOOK

SPRING Meeting BSCDB

JUNE 13, 2019

Château de Colonster, Liège

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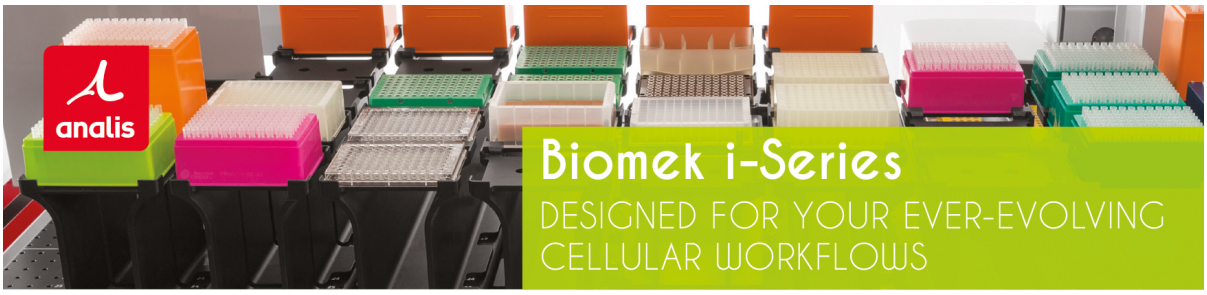

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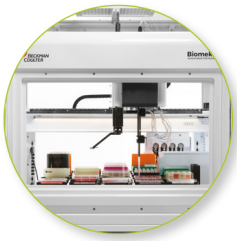

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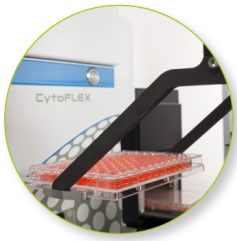

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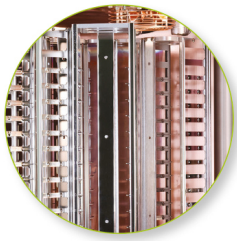

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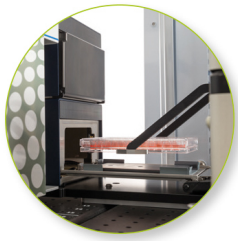

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PROGRAM

8:45 – 9:00 Welcome

Chairpersons: L .NGUYEN – B. LAKAYE

9:00 – 9:30 KLAUS-ARMIN NAVE
"Powering axons: a novel role of oligodendrocytes in brain energy metabolism"

9:30 – 9:35 Analis

9:35 – 9:50 SIMON VERMEIREN
"Prdm12 directs nociceptive sensory neuron development in trigeminal and dorsal root ganglia via distinct mechanisms"

9:50 – 10:20 PATRIK VERSTREKEN
"The origin of sleep dysfunction in Parkinson's Disease"

10:20 – 11:20 Coffee break an Poster session

Chairpersons: L. DELACROIX – M. VOZ

11:20 – 11:50 BENOÎT VANHOLLEBEKE
"Single-cell control mechanism of brain angiogenesis and blood-brain barrier formation"

11:50 – 12:05 LAURA MASSOZ
"Insight into the Molecular Mechanisms of Pancreatic beta Cell Regeneration in Zebrafish"

12:05 – 12:30 Poster teaser

12:30 – 14h30 Lunch and poster session

Chairpersons: P. CLOSE – B. PEERS

14:30 – 15:00 CATHERINE VERFAILLIE
"Using iPSC for disease modelling, drug screening and/or toxicology"

15:00 – 15h15 CAROLINE WATHIEU
"A role for the tRNA-modifying enzyme Elp3 in tuft cell differentiation and in the immune response in the intestine"

15:15 – 15:30 MOHAMAD ASSI
"Citrine-Kras mouse model as a novel tool to elucidate mechanisms of Kras-driven tumorigenesis"

15:30 – 15:45 RONALD POUYO
"Functions of NEDD4-2 in the auditory portion of the inner ear"

15:45 – 16:30 Coffee break and poster session

Chairpersons: A. CHARIOT – B.MALGRANGE

16:30 – 17:00 MATHIAS HEIKENWÄLDER
"On the role of innate and adaptive immune cells in primary cancer formation and metastasis"

17:00 – 17:30 Poster prize and concluding remarks

17:30 Farewell drinks and BSCDB Board meeting

Abstracts BSCDB Spring Meeting 2019

[1] Autonomous and non-autonomous roles of Pcdh10 in brain development

Aerts Tania, Vanlaer Ria, Seuntjens Eve
Katholieke Universiteit Leuven
Presenting author: Tania Aerts

Protocadherin10 (Pcdh10) is a member of the transmembrane calcium-dependent adhesion protein (cadherin) superfamily. Mutations or copy number variations in the genomic sequence coding for PCDH10 or in its regulatory region have been linked to the occurrence of Autism Spectrum Disorder (ASD) in humans. During embryonic and early postnatal development, Pcdh10 is strongly expressed in the basal ganglia, more specifically in the striatum, pre-optic area, piriform cortex and amygdala of the ventral forebrain. These brain regions contain a balanced mix of projection neurons and interneurons that originate from distinct transient progenitor pools during embryonic development. Furthermore, these structures are part of 'the social brain' and hypothesized to be involved in ASD. In the current project, we specifically knocked out Pcdh10 in all interneurons of the developing brain, by crossing a Dlx5/6-Cre-IRES-GFP mouse with a floxed Pcdh10 mouse. The proliferation, neuronal migration and axonal connectivity in heterozygous and homozygous offspring of embryonic day (E)15.5 was investigated. Our preliminary data indicates that Pcdh10 deletion might affect the proliferation of cells in an autonomous and non-autonomous manner. In addition, the correct allocation of corridor cells seems disrupted.

[2] Temporal patterning of apical progenitors and their daughter neurons in the developing neocortex

Gulistan Agirman, Ludovic Telley, Julien Prados, Nicole Amberg, Sabine Fièvre, Polina Oberst, Giorgia Bartolini, Ilaria Vitali, Christelle Cadhilac, Simon Hippenmeyer, Laurent Nguyen, Alexandre Dayer, Denis Jabaudon
Université de Liège
Presenting author: Gulistan Agirman

During corticogenesis, distinct subtypes of neurons are sequentially born from ventricular zone progenitors. How these cells are molecularly temporally patterned is unknown. We used single-cell RNA sequencing at high temporal resolution to trace the lineage of the molecular identities of successive generations of apical progenitors (APs) and their daughter neurons in mouse embryos. We identified a core set of evolutionarily conserved, temporally patterned genes that drive APs from internally driven to more exteroceptive states. We found that the Polycomb repressor complex PRC2 epigenetically regulates AP temporal progression. Embryonic age–dependent AP molecular states are transmitted to their progeny as successive ground states, onto which essentially conserved early postmitotic differentiation programs are applied, and are complemented by later-occurring environment-dependent signals. Thus, epigenetically regulated temporal molecular birthmarks present in progenitors act in their postmitotic progeny to seed adult neuronal diversity.

[3] Citrine-Kras mice as a novel model to elucidate mechanisms of Kras-driven tumorigenesis

Mohamad Assi, Jean-Nicolas Lodewyckx, Younes Achouri, Claude Gérard, Nicolas Dauguet, Isabelle Houbracken, Yves Heremans, Ilse Rooman, Luc Bouwens, Frédéric Lemaigre, Patrick Jacquemin
Université catholique de Louvain
Presenting author: Mohamad Assi

Kras expression and cellular localization cannot be detected on tissue sections, given the lack of reliable antibodies; thus, important information about Kras regulation are still missing. To address this issue, we developed a mouse model called Citrine-Kras (Cit-K) in which a citrine (GFP variant) gene is fused in frame with Kras gene. Kras could be visualized using anti-GFP antibodies. We showed that the Cit-K fusion protein is present in the pancreas and is able to bind GTP (active). Unexpectedly, in normal adult pancreas, ~40% of acinar cells expressed Kras at the plasma membrane (PM), without activating Erk and Stat3 pathways. To identify how Kras is regulated during pancreatic cancer initiation, we crossed mice bearing KrasG12D mutation, in acinar cells, with the Cit-K model and we induced pancreatitis to allow the formation of neoplastic lesions. In pancreatitis condition, we observed enrichment of Kras near the PM, activation of the ERK and Stat3 pathways and increased expression of Rce1 and ICMT enzymes, necessary for Kras targeting to PM. Additionally, experiments including mass spectrometry on immunoprecipitated Ras and RNA-Seq on acinar cells, identified endocytosis/endosomes as a potential signaling platform for Kras during pancreatitis. Our Cit-K model uncovered unanticipated regulations of Kras subcellular localization.

[4] Discovery analysis of cancer-relevant genes uniquely expressed in dedifferentiated human acinar cells and not in duct cells

Elyne Backx, Elke Wauters, Mathias Van Bulck, Jonathan Baldan, Zhidong Ling, Luc Bouwens, Patrick Jacquemin, Isabelle Houbracken, Ilse Rooman
Vrije Universiteit Brussel
Presenting author: Elyne Backx

Pancreatic acinar cells can dedifferentiate and acquire ductal characteristics, which is critical in tumor development. Nevertheless, duct cells themselves are less prone for development of pancreatic cancer (PC) than dedifferentiated acini. We aimed to clarify which genes are unique for dedifferentiated acini. For this, mixed exocrine preparations of acinar and duct cells were obtained from human pancreatic donor organs and cultured to induce dedifferentiation. We FACS-purified the dedifferentiated acinar cells and duct cells and compared their expression signature. RNAseq analysis detected 1219 genes unique for dedifferentiated acinar cells (Adj $P<0.01$, log fold changes of ≤ -2). The most differentially expressed transcription factor (log fold change=-6.09; adj. P=1,04-123) encodes for a known oncogene. We confirmed that this transcription factor (TF) is highly expressed in embryonic acinar cells and in chronic pancreatitis where acinar cells dedifferentiate. Additionally, there is a moderate correlation between TF and SOX9, an indispensable factor for PC formation. In vitro and in vivo TF depletion in dedifferentiated acinar cells gives rise to a distinct phenotype. In conclusion, we report here the purification and transcriptional profiling of the two human pancreatic exocrine cell types. We uncovered a transcription factor, important in cancer, that is characteristic of dedifferentiated acinar cells.

[5] Carnosine, a natural dipeptide, inhibits tumor growth and cetuximab resistance in mutated KRAS colorectal cancer cells

J. Bellier, M-J. Nokin, A. Tiamiou, B. Chiavarina, B. Costanza, G. Rademaker, F. Durieux, P. G. Cusumano, O. Peulen, V. Castronovo and A. Bellahcène
Université de Liège
Presenting author: Justine Bellier

Colorectal cancer (CRC) is the third most common cause of cancer-related deaths. In CRC, KRAS mutation is associated with resistance to anti-EGFR targeted therapy such as cetuximab. Increased glucose uptake and utilization are important metabolic features of KRAS-mutated CRC cells. Elevated glycolysis leads to methylglyoxal (MGO) formation and subsequent MGO-Advanced Glycation End-products (AGEs) accumulation, hereafter mentioned as MGO stress. We have previously demonstrated that MGO-adducts are consistently detectable in aggressive human breast and colon cancer. In this study, we explore the role of MGO stress in the mechanisms of drug resistance related to KRAS mutation in CRC. We found that KRAS-G12V mutation is associated with a glycolytic phenotype that consistently leads to an increase of MGO production and MGO-adducts accumulation. We demonstrated that MGO is able to increase AKT activity by glycating Hsp27 heat-shock protein. Our in vivo experiments revealed that carnosine, a potent MGO scavenger, is able to affect KRAS-G12V tumor growth and sensitize KRAS-G12V cells to cetuximab. Our data let us propose MGO stress mediated AKT activation as an additional mechanism of resistance to cetuximab and underscores the importance of taking into account energetic metabolic dysregulation next to tumor genotyping when stratifying CRC patients prior to EGFR-targeted therapy.

[6] ATAT1-enriched vesicles promote microtubule acetylation via axonal transport

Aviel Even, Giovanni Morelli, Loic Broix, Silvia Turchetto, Chiara Scaramuzzino, Frédéric Saudou, Miguel Weil, Laurent Nguyen
Université de Liège
Presenting author: Loic Broix

Microtubules are polymerized dimers of α- and β-tubulin that underlie a broad range of cellular activities. Acetylation of α-tubulin by the acetyl-transferase ATAT1 modulates microtubule dynamics and functions in neurons. However, it remains unclear how and why this enzyme acetylates microtubules over long distances in axons. Here, we show that loss of ATAT1 impairs axonal transport in neurons and cell free motility assays confirm a requirement of tubulin acetylation for proper bidirectional vesicular transport. Moreover, we demonstrate that the main cellular pool of ATAT1 is transported at the cytosolic side of neuronal vesicles that are moving along axons. Altogether, our data suggest that axonal transport of ATAT1-enriched vesicles is the predominant driver of α-tubulin acetylation in axons.

[7] Role of methylglyoxal stress in breast cancer progression and metastasis

M. Caprasse, J. Bellier, M-J. Nokin, A. Tiamiou, O. Peulen, V. Castronovo and A. Bellahcène
Université de Liège
Presenting author: Maurine Caprasse

Growing interest in cancer energy metabolism, and in particular the so-called glycolytic switch, points to methylglyoxal (MGO) as a new oncometabolite which accumulation sustains specific tumoral functions. MGO is a very reactive dicarbonyl with high protein glycating capacity, leading to the formation of advanced glycation end products (MGO adducts). Our previous studies have demonstrated that MGO stress triggers enhanced tumor growth and metastasis in vivo. To better dissect the role of MGO during tumor progression, we used MMTV-PyMT murine mammary cancer model that mimics human tumor progression and metastasis. We demonstrate for the first time a cytoplasmic accumulation of MGO adducts in cancer cells, which increased significantly from adenoma to late carcinoma lesions. Our preliminary in vitro data indicate that PyMT tumors-derived cancer cells that have undergone epithelial to mesenchymal transition (EMT) present a higher MGO stress profile when compared with their epithelial counterpart. In vivo, we showed that carnosine, a natural MGO scavenger, significantly reduced spontaneous tumor growth and lung metastasis in PyMT transgenic mice. Taken together, our results suggest an important role for MGO stress in breast cancer progression and give a promising insight in cancer therapy through the use of potent MGO scavenger molecules such as carnosine.

[8] Retinal degeneration and retinal pigment epithelial cell abnormalities in a mouse model with peroxisomal β-oxidation deficiency

Yannick Das, Daniëlle Swinkels, Stefan Vinckier, Marc Fransen, Paul P. Van Veldhoven, Myriam Baes
Katholieke Universiteit Leuven
Presenting author: Yannick Das

The recurrent retinal pathology in peroxisomal disease patients highlights the importance of peroxisomes in the retina. Via β-oxidation, peroxisomes are involved in the metabolism of very long chain and polyunsaturated fatty acids, which are enriched in the photoreceptor outer segments (POS). However, the specific role of this pathway in the retina remains to be elucidated. Here, the retinal phenotype in a mouse model with deficient peroxisomal β-oxidation (MFP2 knockout mice) is characterized. At the age of 8 weeks, MFP2 knockout mice showed reduced retinal function, which was accompanied by shortening of the POS and apoptotic death of photoreceptors. In addition, RPE cells protruded into the POS layer and showed progressive loss of the typical hexagonal shape. Moreover, in these cells lipid droplets accumulated. The RPE abnormalities seem to have an impact on their function, as suggested by a reduction in the level of RPE65, an enzyme involved in the visual cycle, and TEM analysis that is suggestive of a decrease in phagolysosomal activity. In conclusion, our data reveal that defects in peroxisomal β-oxidation affect photoreceptors as well as RPE cells, both morphologically and functionally. In addition, the MFP2 knockout mouse model is an attractive model to perform future mechanistic studies.

[9] Defining the mechanisms of selective mRNA translation regulation during resistance of melanoma to targeted therapies.

Najla El Hachem & Pierre Close
Université de Liège
Presenting author: Najla EL HACHEM

Emerging evidences indicate that tRNA expression is modulated during tumorigenesis and correlates with changes in mRNA translation of genes with specific codon usage. Previous research in our laboratory uncovered the key role of enzymes catalyzing the wobble uridine tRNA modification in cancer through a codon-dependent regulation of selective mRNA translation and the establishment of specific oncoproteomes. It is now becoming clear that tRNA dynamics contribute to key aspects of cancer biology. These evidences also revealed the importance of mRNA codon usage and specific mRNA translation in cancer development and drug resistance. However, the molecular mechanisms linking mRNA codon usage and tRNA dynamics to selective mRNA translation and specific proteome expression remain poorly understood. In this study, we combine systematic codon usage analysis of mRNA along with tRNA sequencing and quantitative proteomics to define new mechanisms underlying therapy resistance in melanoma. Analysis of potential correlations between the codon enrichment analysis and tRNA expression uncovered the importance of the GTG (Val) and GAC (Asp) codons and the corresponding tRNA-Val-(CAC) and tRNA-Asp-(GTC) in resistant cells. Moreover, the expression of the valine tRNA synthase (VARS) is correlated with poor prognosis in melanoma patients and its protein levels was found upregulated in resistant melanoma cells. Importantly, VARS depletion resensitized resistant melanoma cells to MAPK-based therapy, while it had no significant effect on untreated cells. In vivo, the depletion of VARS synergized with MAPK-therapy to induce tumor shrinkage of resistant melanoma. Further experiments are ongoing to identify VARS translational targets and to further define the mechanisms underlying the protective effect of VARS in melanoma resistance to targeted therapies. Taken together, these data underline the potential role of VARS in melanoma development and during resistance to therapy.

[10] Unveiling the alcohol-dependent alterations of local translation in the prefrontal cortex during adolescence

Laguesse S, Van Hees L, Nguyen L
Université de Liège
Presenting author: Sophie Laguesse

During adolescence, the brain undergoes intense maturation, particularly in the frontal areas. The prefrontal cortex (PFC) is implicated in executive functions and its immaturity in adolescents is associated with lack of inhibitory control, increased impulsivity and desire of risk-taking. Studies have suggested that Adolescent Alcohol Exposure (AAE) may interfere with the ongoing maturation of frontal brain circuits, leading to profound long-lasting consequences on PFC function. In addition, clinical studies have shown that AAE significantly increases the risk of developing psychiatric and behavioral disorders later in life, including alcohol addiction. However, the cellular and molecular mechanisms underlying the alcohol-induced defects in PFC maturation are still poorly understood. Alcohol addiction is considered as a maladaptive form of learning and memory, “usurping” the molecular mechanisms underlying those processes, including synaptic plasticity, which depends on the local translation of mRNAs at synaptic sites. We previously reported in adult mice that excessive alcohol consumption modifies synaptic protein composition in brain regions associated with the mesocorticolimbic pathway, promoting the development of alcohol addiction. Here by using a mouse model of voluntary adolescent binge drinking , we report that alcohol intake during adolescence modulates the activity of local translation regulators in the PFC and leads to long-lasting behavioral impairments in adulthood, such as increased anxiety and alcohol intake as well as reduced cognitive performances.

[11] A-type lamin status determines susceptibility to and recovery after compression-induced nuclear envelope rupture

Ana Leal, Joke Robjins, Guilherme Nader, Juan Manuel Garcia, Matthieu Piel, Winnok H. de Vos
Universiteit Antwerpen
Presenting author: Ana Leal

The cell nucleus is supported by a network of nuclear lamins. Mutations in the LMNA gene, which encodes A-type lamins, cause a variety of diseases called laminopathies. We have discovered that nuclei from laminopathy patient cells undergo repetitive, non-lethal ruptures of the nuclear envelope (NERs) [1]. Migrating cancer cells experience a similar process, suggesting it represents a broad-spectrum pathogenic mechanism [2,3]. To understand the contribution of A-type lamin defects to the susceptibility to NERs, we have previously developed cell lines that either do not express A-type lamins (LMNA KO), or that produce a mutant prelamin A isoform (ZMPSTE24 KO). Our first results revealed that absence of A-type lamins results in a higher NER frequency than in control cells, whereas prelamin A accumulation does not [4]. Afterwards we asked if the A-type lamin status would render cells more susceptible to compression-induced NER. After being compressed, we could observe that both LMNA KO and ZMPSTE24 KO cells experienced elongated recovery times after NER, suggesting that both conditions compromise NE repair. As of this moment, we are exploiting the multiwell-confinement approach that compresses cells in suspension between two surfaces down to a well-defined height of 4 µm [5]. NERs are being visualized in living cells by transient translocation of a nuclear-localized fluorescent protein (mCherry-NLS) to the cytoplasm and quantified using a home-written image analysis pipeline. By using the new confinement system, we will try to validate the previous results obtained in the lab and assess if they are consistent. We hope to implement a robust approach to quantify immediate downstream effects and long-term responses in NERs kinetics. Ultimately, this will lead to the identification of new drug targets to reduce or exploit NERs in disease treatment.

[12] Maternal Zic2 regulates the organizer formation by epigenetically controlling Wnt/ βcatenin signaling.

Azonpi Lemoge A. P. , Tchouate Gainkam O., Houtmeyers R. , Van den Bosch B. , Etlioglu E., Deschamps W. , Butera Y. 1,3, Arkell R. 4 , Mutesa L. 3 , Tejpar S. , and Souopgui J. 1,
Université Libre de Bruxelles
Presenting author: Arnaud Lemoge

ABSTRACT The Spemann organizer is an essential signaling centre in Xenopus axis formation. The establishment of Spemann organizer is due to the dorsal accumulation of nuclear β-catenin under the influence of cytoplasmic determinants displaced by fertilization. It has been reported that β-catenin binds to TCF/LEF and recruits the methyltransferase enzyme Prmt2 in order to modify the chromatin structure there by inducing the expression of dorsal organizer genes. Zic2 is a member of the Zinc finger of cerebellum family. In human and mice, the lack of functional Zic2 provokes a defective node and holoprosencephaly. Zic2 can modulate key organizer gene cascades such as canonical Wnt/β-catenin and TGF-β/Nodal signaling, but its precise role in the organizer/node development remains unknown. Here, we report using animal cap assay and ChIP experiments, that the maternal Zic2 is required for the canonical Wnt signaling and it acts as a pioneer factor. We provide evidence that maternal Zic2 modulates Prmt2-mediated epigenetic bookmarking of crucial organizer genes before the midblastula transition while zygotic Zic2 controls homeostasis of both Wnt/β-catenin and TGFβ/Nodal gene targets during organizer formation. Keywords: Zic2, epigenetic, H3K4me3 marks, Canonical Wnt, TGF-b/Nodal, Spemann organizer, Xenopus laevis

[13] Implication of translation reprogramming in tumour microenvironment and breast cancer metastasis

Lenelle A., Duysens G., Roncero A., Marichal T., Desmet C., Chariot A. and Close P
Université de Liège
Presenting author: adrien lenelle

Metastatic disease remains the primary cause of death for patients with breast cancer. Tumours maximize their chance of metastasizing by evoking a systemic inflammation that culminates in expansion and polarization of a specific neutrophil population. As such, neutrophils have recently been pointed as the main component and driver in establishment of lung and lymph node metastasis in breast cancer. Prior the development of metastasis in the lungs, the quantity of neutrophils increases and it is believed to favor the development of metastasis in the lungs. Also, in a cancer context, recent data show that neutrophils undergo a gene expression remodeling and are polarized into tumour-associated neutrophils (TANs), which are now considered as a distinct population than normal neutrophils (NNs). Importantly, our laboratory recently highlighted the importance of wobble uridine tRNA modification (U34-TM) in the establishment of specific proteomes that sustain WNT-dependent intestinal tumour initiation and breast cancer metastasis. Strikingly, we found that U34-TM enzymes (i.e. Elp3, Alkbh8 and Ctu1/2) are up-regulated in neutrophils extracted from mice bearing breast tumours, as compared to neutrophils from naïve mice. In this project we hypothesized that U34-TM sustains translation reprogramming in neutrophils during breast cancer. We generated an U34-TM-loss of function model in neutrophils by crossing the Elp3lox/lox mouse strain with the neutrophil specific Mrp8-CRE strain. Our results show that the absence of Elp3 strongly impacted the number of neutrophils in the lung and the spleen of tumour bearing mice. Importantly, this is correlated with a dramatic decrease in metastases burden in PyMT mice upon Elp3 deficiency in neutrophils. Surprisingly, the loss of Elp3 in neutrophils led to a large decrease in the number and the size of the primary breast tumors. These results indicate that U34-TM play a key role in neutrophils during breast cancer development. Our future work will be dedicated to dissect the immune cells regulation leading the antitumoral effect, and to identify the translation changes taking place in neutrophils during breast cancer development.

[14] Characterization of Oligodendrocyte Precursor Cell Migration During Corticogenesis

Fanny Lepiemme, Carla G. Silva, Laurent Nguyen
Université de Liège
Presenting author: Fanny Lepiemme

During embryogenesis, oligodendrocyte precursor cells (OPCs) are derived from distinct progenitors of the ventral forebrain. The first cohort is generated in the medial ganglionic eminence (MGE) and preoptic area (POA) and starts migrating at E11.5. Two additional waves of migrating OPCs are born around E16.5 in the lateral ganglionic eminence (LGE) and P0 in the pallium, respectively. While their origins have been well described, their migration mode remains poorly understood. By combining real-time imaging with histological analyses, we show that embryonic OPCs have distinct migration parameters (e.g. migration speed and migration pattern) as compared to cortical interneurons that are also generated in the subpallium and that migrate concomitantly with OPCs. This suggests that the cell migration modes adopted by immature glia and interneurons are distinct and partially encoded in their respective progenitors. Moreover, the migration properties of OPCs change as their morphological complexity increases and the migration pattern adopted by OPCs generated at the same developmental stage varies slightly, suggesting that interaction with distinct components of the environment influence their migration properties.

[15] Identification of Signaling Pathways Stimulating Beta Cell Regeneration

Laura Massoz, David Bergemann, Célia Reynders, Arnaud Lavergne, Claudio Carril Pardo, Jordane Bourdouxhe, Bernard Peers, Marianne Voz, Isabelle Manfroid .
Université de Liège
Presenting author: Laura Massoz

Type 1 Diabetes is characterized by destruction of the insulin-producing pancreatic beta-cells. Beta-cell replacement constitutes a promising alternative to replenish the pancreas with beta-cells. One way to achieve this goal would be via beta-cell neogenesis. This has been shown to be possible from different pancreatic cell types. Still, mammals show limited regenerative capabilities. In contrast, zebrafish has the ability to regenerate its tissues and notably its beta-cells. We previously showed that pancreatic ductal cells in the adult zebrafish display characteristics of pancreatic progenitors that give rise to beta-cells. To better understand the molecular mechanisms, transcriptomic profiling has been performed on the pancreatic ducts and revealed differentially expressed genes enriched in “Cell cycle”, “Calcium”, “Notch”, “PI3K” pathways. Using larvae to test pharmacological inhibitors targeting key effectors of these pathways, we showed that inhibition of Calcineurin (CaN), a protein phosphatase of the Calcium pathway improves beta-cell regeneration. To dissect the molecular role of CaN in beta-cell neogenesis, we induced formation of beta-cells from the ducts by inhibition of the Notch pathway. By inhibiting CaN and Notch pathways, we saw that CaN inhibition potentiates the effect of Notch inhibition. These data suggest that CaN modulates cell cycle properties of the pancreatic-progenitors during regeneration.

[16] CRISPR-NS-ID: an in vivo CRISPR/Cas9 negative selection screen reveals EZH2 as a druggable dependency factor in a genetic desmoid tumor model

Thomas Naert, Tom Van Nieuwenhuysen, Joanna Przybyl, Suzan Demuynck, Dieter Tulkens, Marnik Vuylsteke, Sven de Grande, Rivka Noelanders, Dionysia Dimitrakopoulou, David Creytens, Matt van de Rijn, Savvas N. Savvides and Kris Vleminckx
Universiteit Gent
Presenting author: Thomas Naert

Identification of genetic dependencies in cancer is pivotal to the elucidation of novel therapeutic strategies to improve prognosis prospects for cancer patients. Unfortunately, in vivo identification of genetic dependencies has long relied on expensive and time-consuming breeding of genetically engineered animal models. We developed a new methodology called CRISPR/Cas9-mediated Negative Selection Identification of genetic Dependencies (CRISPR-NS-ID) that allows in vivo elucidation of cancer cell vulnerabilities in genetic cancer models. Our methodology hinges on the phenomenon that for a genetic dependency there is an incapability for recovering tumors carrying biallelic frameshift mutations in this dependency gene. We describe the binomial statistics to ascertain this negative selection pressure via the deviation between tumor-recovered CRISPR/Cas9 scarring patterns and CRISPR/Cas9 scarring patterns under absence of selection. We employ CRISPR-NS-ID to identify ezh2 and creb3l1 as genetic dependencies in desmoid tumors occurring in a Xenopus tropicalis tumor model and demonstrate the promise of EZH2 inhibition as a new therapeutic strategy for desmoid tumors. We believe CRISPR-NS-ID establishes a new methodology for rapid identification of genetic dependencies in monoclonal disorders with wide adaptability to other model systems and organisms. Preprint Available @ BioRxiv DOI: 10.1101/595769

[17] Modeling epilepsy and mental retardation limited to females (EFMR) in the dish and in vivo

Pancho Yanza A., Mitsogiannis M., Dalla Vecchia M., Staes K., Van Laer R., Van Roy F., Dedecker P., Schermer B. and Seuntjens E.
Katholieke Universiteit Leuven
Presenting author: Anna Gabriela Pancho Yanza

Protocadherin 19 (PCDH19) is a transmembrane protein whose gene is located on the X-chromosome. Mutations in PCDH19 cause epilepsy and mental retardation limited to females (EFMR). Most strikingly, solely woman show severe symptoms, while hemizygous carrier men are spared. Epilepsy has been linked to defective neuronal migration during embryonic brain development. It seems that mosaic absence of PCDH19, caused by random X-inactivation, plays a central role in the development of EFMR. We first assessed Pcdh19 expression during mouse brain development and found confined expression within the neocortex and regions from which interneurons originate. To model the mosaic imbalance in Pcdh19 expression, we used Pcdh19 knockdown and overexpression in living slices and detected perturbed cortical interneuron migration. Fascinatingly, our Pcdh19 overexpression model suggested increased neuronal death. Hence, we further investigated the molecular cause. To this end we created and validated a series of GFP-tagged deletion constructs of Pcdh19. Early and late apoptosis induced by overproduction of the complete protein or its subdomains was assessed in the Neuro2A cell line. A significant increase in cell death could only be detected for the complete protein as well as Pcdh19 lacking the intracellular domain. This suggests apoptosis as a new mechanism for EFMR.

[18] Functions of NEDD4-2 in the auditory portion of the inner ear

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Presenting author: Ronald POUYO

Hearing loss is the most common neurosensory disorder with more than 466 million people affected worldwide. In most cases, hearing loss is caused by the dysfunction or the loss of the sensory cells (the hair cells) and/or their afferent neurons (spiral ganglion neurons) in the cochlea. To date, many genetic mutations have been associated with congenital deafness or early-onset hearing loss, allowing for the discovery of genes involved in the development or maintenance of the cochlea. A recent report identified deafness-associated mutations in the gene encoding the E3 Ubiquitin-ligase NEDD4L in humans, we plan to uncover its implication in cochlear development and function. We first characterized the spatio-temporal expression of NEDD4-2 by in situ hybridization, confirming the transcripts presence during the embryonic stages of cochlear development. To decipher Nedd4-2 roles during mouse cochlear development, we generated conditional knockout animals and our first results suggests an early degeneration of hair cells and their innervating spiral ganglion neurons following a gradient from the basal to the apical turn of the cochlea. This phenotype starts around P45 until P90, stage were we observed a total hair cell loss. Our results already suggest a major contribution of the ubiquitin ligase NEDD4-2 in the maintenance of the adult cochlea integrity and we will combine mouse genetics with cellular and molecular analyses to decipher the role of Nedd4-2, which may provide future therapeutic perspectives against hearing loss.

[19] Myoferlin controls pancreatic cancer metastases through oxidative phosphorylation

Gilles Rademaker , Brunella Costanza , Sandy Anania , Ferman Agirman , Naima Maloujahmoum , Emmanuel Di Valentin , Jean Jacques Goval , Akeila Bellahcène , Vincenzo Castronovo and Olivier Peulen

Université de Liège
Presenting author: Gilles Rademaker

Pancreatic cancer in one of the deadliest cancers with an overall survival of 5%. Most of PDAC are discovered at an advanced stage mainly due to the difficulty to diagnose this pathology. Metastasis is the first cause of death in pancreatic cancer patients. Understanding the mechanism of tumor metastases and dissemination remains consequently a priority. Mitochondrial oxidative phosphorylation has been shown to be implicated in tumor dissemination. Here we show that myoferlin, a protein overexpressed in pancreatic cancer, is involved in metastases and cell migration. In vitro, we show that cells expressing high myoferlin level have higher migratory potential than cells characterized by a low myoferlin abundance. Moreover, we demonstrate that myoferlin silencing leads to a migration decrease associated to a reduction of mitochondrial respiration. In vivo selection of liver-tropic pancreas cancer cells demonstrate the relation between high myoferlin expression, oxidative phosphorylation and metastases. We consider myoferlin as a valid potential therapeutic target recently targeted by a pharmacological compound.

[20] Uncovering the U34 tRNA modifying enzymes target proteome and its impact in human disease.

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Presenting author: Francesca Rapino

Regulation of mRNA translation is of key importance for tumor cell adaptation to stress, such as oncogenic transformation, metastasis or resistance to therapy. Despite their universal function, variation in tRNA expression profiles is now believed to impact the protein expression landscape. While the role and regulation of tRNA levels in cancer is still under debate, some chemical modifications occurring on tRNAs have been directly linked to cancer development. Interestingly, the translational efficiency, which relies on the tRNA-mRNA pairing, varies between codons leading to bias in codon usage. To improve wobble base recognition, chemical modifications of tRNAs or base-editing may occur in position 34 of tRNAs. Among others, we showed that the mcm5s2 modification of the uridine 34 (U34-TM) of some tRNAs increases the decoding capability of AAA, GAA and CAA codons. Recently, we uncover the pivotal role of the U34-TM pathway in promoting codon-specific translation reprogramming and acquired resistance of melanoma to targeted therapy: the impairment of U34-TM enzymes sensitizes resistant melanoma cells to BRAF inhibitors by codon-dependent translational regulation of HIF1α and subsequent impairment of glycolysis. Therefore, the acquired resistance to anti-BRAF therapy is associated with high levels of U34-TM enzymes and HIF1α in melanoma. The extraordinary specificity of the U34-TM enzymes addiction, prompt us to study the core features needed to be a translational target of this pathway. The aim is to predict susceptibility of mRNAs towards the U34-TM therefore highlighting pathological contexts were these enzymes can be targets for therapy. Using a combination of bioinformatics and proteomic approaches we propose a model where U34 codon usage, folding and aggregation propensity can confidently predict U34-TM targets and relevant diseases where their impairment would be of therapeutic relevance.

[21] Mechanism of action of NeuroD1 in zebrafish intestine

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Presenting author: Anne-Sophie Reuter

ARP/ASCL factors are key determinants of cell fate specification and differentiation in a wide variety of tissues, notably in the digestive system. Cross-species comparison amongst vertebrates highlighted that the identity of these determinants depends on the organ but also on the species. As zebrafish is a good and easy model to perform phenotypic rescue experiments, we tested in this model whether expression of other members of the ARP/ASCL family could rescue the intestinal secretory defects of ascl1a-/- mutant. We showed that any ARP/ascl factor is able to initiate the first step of the secretory cascade but the subsequent step(s) of the endocrine differentiation program requires Neurod1, as it is the only one able to rescue enteroendocrine cells while other ARP/Ascl tested only rescue goblet intestinal cells. By constructing hybrid proteins between Ascl1a and Neurod1, we could highlight a domain, highly conserved in all NeuroD subfamily members but not present in the atonal, neurog and ascl members, which is necessary and sufficient to rescue the enteroendocrine cells. By comparing mutant and native forms of NeuroD1 regarding their targets and their interacting partners, we will be able to understand to which extend this conserved domain is responsible for NeuroD1 specificity of action.

[22] Novel U34-tRNA modification targets highlight a new therapeutic approach in triple negative breast cancer.

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Université de Liège
Presenting author: ANA MARIA RONCERO SANCHEZ

Translation of mRNAs into proteins is one of the key processes in cell homeostasis. Recent evidences demonstrated that reprogramming of mRNA translation plays a key role in cancer development and drug resistance. Modification at the wobble uridine (U34) of tRNAs is required for specific codon decoding during mRNA. Our lab previously revealed the importance of the enzymes responsible for wobble U34-tRNA modification in cancer development and resistance to therapy, by sustaining the synthesis of specific oncoproteins. In this work, we aimed to define the extent of mRNA species that require U34-tRNA modification during translation and to highlight specific pathological contexts that rely on U34-tRNA modification. Using a combination of bioinformatic analysis, ribosome IP, proteomic approaches and in vivo experiments, we have identified new targets mRNAs of the U34-tRNA modification pathway. We found that the depletion of U34-enzymes leads to the misfolding of the corresponding protein products and their incorporation into protein aggregates for degradation. Moreover, by clustering analysis, we uncovered that triple negative breast cancers (TNBC) develop a specific addiction towards the U34-tRNA modification pathway to sustain cell viability and survival. In conclusion our results uncover new U34-tRNA modification targets and indicate that U34-enzymes may represent therapeutic targets in triple-negative breast cancers.

[23] Design of a microfluidic chip for neurovascular unit organoid

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Presenting author: Idris Salmon

The high failure rate of drugs for brain diseases such as Alzheimer's is a well-known problem in the pharmaceutical sector. This problem persists despite the fact that prospective drugs are extensively studied. One major problem is the current lack of good in vitro blood brain barrier (BBB) models. Recent advances in both the stem cell and engineering field create new possibilities to generate an all human highly biomimetic BBB on a microfluidic chip. Here, we use an interdisciplinary approach to design a microfluidic chip for growing a BBB on-chip, with the goal of generating iPSC-derived vascularised brain organoids. Our custom designed and printed microfluidic chip is a scaffold that can be perfused by culture media and is composed of a central neural compartment surrounded by a vascular compartment. The orientation of both compartments mimics the developing avascular brain before it gets vascularized by surrounding vasculature. Ongoing studies focus on developing a co-culture model of brain organoids together with iPSC-derived endothelial cells and pericytes in spatially defined configurations, in order to recapitulate the process of intraneural vessel sprouting and BBB formation. Once established, this all human in vitro BBB model will contribute to drug screening assays in increasingly biomimetic disease modelling.

[24] Implication of U34 tRNA modification in lung cancer development

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Université de Liège
Presenting author: Sebastian Schmitz

Dynamic qualitative and quantitative changes in proteome occur during tumorigenesis, metastasis and resistance to therapies. Recently, our work showed that U34-TM promotes a codon-specific translation reprogramming in melanoma cells that sustains resistance towards targeted therapies. In addition we found that the mTORC2 complex (enclosing RICTOR) is the kinase directly responsible for U34-TM upregulation . A recent study demonstrated that amplification of the RICTOR gene occurs in 11% of lung cancers as the only relevant genomic alteration. In this frame, the goal of my project is to study the implication of wobble U34-tRNA modification upregulation in lung cancer progression and translation regulation. Importantly we performed large scale IHC analyses in patient biopsies of lung adenocarcinoma and we found that the expression of Rictor, strongly correlates with the expression of the U34-TM enzymes. Our preliminary results show that mTORC2, but not mTORC1 inhibition, leads to elongator downregulation. Also upregulation of Rictor using a model for intrinsic mTORC2 activation shows, that increased RICTOR expression leads to mTORC2 activation and correlates with high levels of the U34-TM enzymes. Taken together our preliminary results, we identified the oncogenic pathway that promotes U34-TM regulation in lung cancer. Understanding the contribution of this new enzymatic cascade in cancer proteome remodelling will highlight fundamental mechanisms underlying cancer cell adaptation and may reveal new therapeutic opportunities for anti-cancer treatment.

[25] Impact of codon-biased translational regulation in melanoma immune response.

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Presenting author: Christian Seca

Malignant melanoma contributes in larger part to patient death due to skin cancer. The breakthrough discoveries on the role of the immunity system in tumor development led to new and effective treatments of melanoma patients. Nevertheless, poor response and acquired resistance limit the benefits

of immunotherapy. Recently, we uncovered the key role of codon bias translational regulation in melanoma in response to targeted therapy. Impairment of the enzymatic cascade that catalyzes tRNAs' uridine 34 (U34-TM) mcm5s2 modification strongly limits melanoma resistance to BRAF inhibitors. In this project, we postulate that codon specific mRNA translation reprogramming occurs in melanoma cells during exposure to immune pressure and it is crucial for melanoma immunogenicity. Our preliminary results showed that U34-TM enzymes depletion in B16 cells enhanced melanin production, and induced interferon gamma gene signature -a key mediator of immune response in cancer. Moreover, the lack of U34-enzymes strongly reduced tumor growth in immunocompetent mice, but no effect was observed in immunocompromised mice. These data suggest that U34-enzymes play a role in the anti-tumoral immune response in melanoma. Our future work will be dedicated to understand the role of U34-enzymes in melanoma immunogenicity. We propose to uncover new regulators of codon bias translational regulation in proteome rewiring upon immune-response in melanoma. To this end, we will assess the codon usage of the translatome of melanoma grown in immunocompetent mice, their tRNA expression and modification status. Taken together this project will indubitably shed light on the role of codons specific translation regulation in immune response and will pinpoint new therapeutic targets for immunotherapy.

[26] The X-linked trichothiodystrophy-causing gene RNF113A links the spliceosome to cell survival upon DNA damage

Kateryna Shostak, Zheshen Jiang, Benoit Charloteaux, Yvette Habraken, Lars Tharun, Xu Xinyi, Hong Quan Duong, Andrii Vislovukh, Pierre Close, Alexandra Florin, Florian Rambow, Jean-Christophe Marine, Reinhard Büttner and Alain Chariot
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Presenting author: Kateryna Shostak

Prolonged cell survival, a hallmark of cancer cells, can occur through the expression of specific protein isoforms generated by alternate splicing of mRNA precursors. How alternate splicing regulates tumor development and the acquired resistance to targeted therapies in cancer remain poorly understood. Here we show that RNF113A, whose loss-of-function causes the X-linked trichothiodystrophy, is overexpressed in clinical cases of lung cancer, is induced by Cisplatin and protects from Cisplatin-dependent cell death. RNF113A acts as a spliceosome subunit and a RNA-binding protein to properly splice multiple candidates involved in cell survival. RNF113A also promotes the polyubiquitination of many candidates such as USP9X involved in DNA repair. RNF113A deficiency triggers cell death upon DNA damage through multiple mechanisms, including apoptosis via the destabilization of the prosurvival protein MCL-1, ferroptosis due to enhanced SAT1 expression and increased production of ROS due to less Noxa1 expression. The inhibition of RNF113A circumvents the resistance to Cisplatin and to BCL-2 inhibitors through the destabilization of MCL-1, which thus defines spliceosome inhibitors as a therapeutic approach to treat tumors showing acquired resistance to specific drugs due to MCL-1 stabilization.

[27] The impact of culture conditions on the phenotype of human fetal astrocytes

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Astrocytes make up a crucial component of the blood brain barrier and have been attributed many supporting and regulatory roles in the brain. Much literature on astrocyte physiology and pathology stems from rodent primary culture, cancer cell lines and more recently also iPSC-derived astrocytes. However, the golden standard remains human astrocytes from resected brain material. As this material is precious and difficult to produce, we set out to investigate whether it is possible to produce an in vitro astrocyte culture from commercial fetal astrocytes (Sciencell SC1800) that resembles primary astrocytes derived from the human cortex. qPCR and immunocytochemistry experiments revealed the expression of the commonly used astrocyte markers, such as GFAP, S100B, AQP4 and ALDH1L1. However, to more systematically gauge the nature of the cultured astrocytes we are now performing a transcriptomic profiling of the cell cultures grown under different conditions. We hereby compare the substrate (plastic, glass and silicone substrates coated with laminin of either mouse or human origin) and the growth medium (containing either horse serum, human serum or a serum-replacement). A direct comparison with a previously published dataset of mature human astrocytes (Zhang), will help determining the optimal culture condition, and may also reveal biophysical patterning factors. Reference: Zhang, Y., Sloan, S. A., Clarke, L. E., Caneda, C., Plaza, C. A., Blumenthal, P. D., … Barres, B. A. (2016). Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse. Neuron, 89(1), 37–53. https://doi.org/10.1016/j.neuron.2015.11.0131

[28] Transcriptomic and DNA methylation analysis points to a novel link between dicarbonyl stress and epigenetic regulation in breast cancer

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Presenting author: Assia Tiamiou

Methylglyoxal (MGO) is a very reactive dicarbonyl molecule derived from glycolysis. MGO interacts with DNA, lipids and proteins to form Advanced Glycation Products (AGEs). Our previous studies have demonstrated that MGO glycating stress triggers enhanced tumor growth and metastasis in breast cancer. The glyoxalase system composed of GLO1 and GLO2 enzymes present in all mammalian cells is one of the major defences against MGO stress. We generated stable GLO1-depleted breast cancer cell to induce an endogenous MGO stress. Transcriptomic analysis of GLO1-depleted cells revealed a pro-metastatic MGO signature notably comprising the regulation of invasion and ECM-related genes expression. A comprehensive genome wide methylation analysis performed on GLO1-depleted cells pointed to a significant global hypermethylation. This study aims to explore the potential connexion between the acquisition of MGO dicarbonyl stress pro-metastatic phenotype and changes in the epigenetic machinery. Interestingly, transcriptomic analysis revealed an increase of DNMT3A expression, one of the main de novo DNA methyltransferases, in GLO1-depleted cells. DNMT3A mRNA and protein levels were increased upon exogenous MGO treatment and decreased in presence of MGO scavengers thus confirming the link with MGO stress. Ongoing analyses will help integrating gene expression data with gene methylation status in cancer cells under MGO stress.

[29] NOVEL GENETIC LEUKEMIA MODEL IN XENOPUS TROPICALIS USING A CRISPR/CAS9 BASED MULTIPLEX APPROACH

Dieter Tulkens, Dionysia Dimitrakopoulou, Suzan Demuynck, Thomas Naert, Sylviane Dewaele, Wendy Toussaint, Gert Van Isterdael, Kelly lemeire, Pieter Van Vlierberghe and Kris Vleminckx
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Presenting author: Dieter Tulkens

Acute lymphoblastic leukemia (ALL) is an aggressive tumor entity that is one of the most common childhood malignancies worldwide. Despite our increasing understanding of the genomic defects in human T-ALL, leukemia patients are still treated by high-dose multi-agent chemotherapy, potentially followed by hematopoietic stem cell transplantation. Still, many patients still relapse and present with very unfavorable survival perspectives. Therefore, more effective and less toxic molecular targeted therapies are required. We recently generated a model for T-ALL in the frog Xenopus tropicalis using CRISPR/Cas9. Characterization is done by genotyping experiments showing clonal enrichment of T-cell lymphoblasts in the thymus of leukemic animals. In addition, by phenotyping procedures such as blood staining, flow cytometry, RT-qPCR and histology, we further support the diagnosis of T-ALL. Currently, we are exploiting this model to identify new anchor points for targeted therapy. We use fast and efficient multiplexed gene disruption via CRISPR/Cas9 for the screening of candidate "dependency genes", which encode proteins on which the leukemic cancers cells rely for their proliferative and malignant behavior. The identification of these dependency factors will open the road for novel drug development efforts that could be beneficial for a wider range of hematologic malignancies, as well as solid tumors.

[30] Deciphering the role of tRNAs modifications in local translation at synapses.

Silvia Turchetto, Broix Loic
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Presenting author: Silvia Turchetto

The establishment of functional synaptic connections during cortical development relies on locally synthesized proteins. Transfer RNAs (tRNAs) are key players in the translation of mRNAs into proteins and specific tRNAs require modification of the wobble uridines (U34) to be functionally active. Despite the Elongator complex is a key player in this process, its contribution to the local synaptic proteome and synaptogenesis hasn't been investigated yet. By immunolabeling, we demonstrate that Elongator is expressed in different subcellular compartments of embryonic day (E)14 mouse cortical neurons cultured 7 DIV, including excitatory and inhibitory synapses. Western blotting on synaptosomal extracts isolated from P7 mouse cortices detected the expression of Elp1, Elp3, Alkbh8, and Ctu1, the three enzymes that act in a multi-step reaction to promote tRNA modifications. Of interest, in Elp3-depleted cortical projection neurons (PNs) cultured 7 days in vitro (DIV) we observed a significant decrease of positive puncta for the excitatory synaptic markers PSD95 (postsynaptic) and Vglut1 (presynaptic). Additionally, we report reduced puromycin labelling in their soma and along their dendrites, suggesting reduced total protein synthesis. Taken together, these preliminary data support a possible role for Elongator in local protein synthesis at cortical neuron synapses.

[31] Unveiling the neurogenesis defects induced by prenatal alcohol exposure.

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Presenting author: Laura Van Hees

Prenatal alcohol exposure (PAE) is known to damage the fetal brain and lead to life-long cognitive and behavioral dysfunctions. Alcohol is believed to interfere with the cerebral cortex development in a variety of ways; however, the precise pathophysiological mechanisms underlying alcohol's actions are yet poorly understood. In this study, we use pregnant mice voluntarily drinking high amounts of alcohol throughout pregnancy as a model of Fetal Alcohol Spectrum Disorder, and showed that mice reach blood alcohol concentration levels comparable to those reported in binge-drinking humans. We investigated the alcohol-dependent corticogenesis defects, by analyzing the survival, proliferation, specification and

migration of projection neurons during embryonic development. By using in utero electroporation, we observed delayed neuronal migration in the sensory cortex of alcohol-exposed embryos. We are now studying the different steps of radial migration by using time-lapse imaging in organotypic slices, to precisely define the alcohol-induced defects on radial migration of projection neurons. Moreover, in order to determine whether PAE has a long-term impact on behavior, we investigated tactile sensitivity by using the adhesive removal test. Our preliminary results have shown that alcohol-exposed females exhibit both increased initial contact time (sensory component) and removal time (motor component), compared to control females, but no difference was observed in males, suggesting sex-specific long-lasting impairment of sensory motor cortical regions induced by PAE. We plan to perform additional behavioral tests to evaluate anxiety levels (open-field, elevated-plus maze), and sociability (three chamber test).

[32] A new gerontology model to improve brain repair in an aged environment: The African turquoise killifish

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Presenting author: Jolien Van Houcke

The African turquoise killifish has a compressed lifespan to cope with the extreme habitat of desiccating pools. As a consequence, this teleost fish has many aging characteristics resembling human aging. We choose to validate this novel animal model to unravel the influences of aging on brain repair after injury. The neuroscience field is in a clear need for such a new short-lived vertebrate model that enables studying the aged cellular environment, known to contribute and aggravate the outcome of many brain diseases, including neurodegenerative disease. Since no effective treatments are available for these diseases, it is imperative to invest in developing new strategies that constrain neurodegeneration and allow induction of neuroregeneration throughout the lifespan. We injure the telencephalon to induce neuroregeneration in young and old fish. First data show that regeneration is impaired in the aged killifish brain, with stem cells becoming unresponsive to injury, thereby hampering regeneration as observed in the young telencephalon. Remarkably, most of the proliferating stem cells in young killifish seem to be BLBP negative, a typical marker of radial glia. Ongoing transcriptome and proteome analyses will reveal for which genes/proteins expression should be manipulated in order to rejuvenate the aging stem cell pool and/or environment to reinstall successful regeneration in an aged brain.

[33] Mas-Related G Protein-Coupled Receptor C11 (Mrgprc11) induces visceral hypersensitivity in the mouse colon: a novel target in gut nociception?

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Presenting author: Samuel Van Remoortel

Visceral hypersensitivity is a hallmark of gastro-intestinal (GI) abdominal pain disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). It typically presents as an increased pain sensitivity in the gut, mainly caused by aberrant nociceptive input from the gut towards the spinal cord. Nevertheless, our understanding still remains poor and a better characterization of the underlying drivers of gut nociception is needed, as this could help define novel therapeutic targets for visceral hypersensitivity. Recently, the mouse Mas-related G protein-coupled receptor c11 (Mrgprc11), a GPCR expressed by nociceptive DRG neurons, emerged as a promising target in skin and lung nociception, yet its role in gut nociception is uninvestigated. Here, we discovered Mrgprc11 expression by DRG neurons

innervating the mouse gut and found that receptor activation in the gut of healthy mice induces visceral hypersensitivity. Moreover, from a translational point of view, we found that the human counterpart of Mrgprc11, MRGPRX1, is similarly expressed in human DRG neurons. Overall, these findings expose a novel role for Mrgprc11 in gut nociception, but more importantly, warrant further research on exploring the therapeutic potential of Mrgprc11 and its human counterpart in GI abdominal pain disorders.

[34] Prdm12 directs nociceptive sensory neuron development in trigeminal and dorsal root ganglia via two distinct mechanisms

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Presenting author: Simon Vermeiren

Mutations in PRDM12 have recently been identified in patients with Congenital Insensitivity to Pain (CIP) indicating a putative role for this epigenetic modifier in pain sensing. Here we show that Prdm12 is expressed selectively in developing somatosensory neural precursors and differentiating nociceptors, the specialized neurons sensing damaging stimuli, and that the development of nociceptors is selectively eliminated in Prdm12 null and conditional mutant mice. Mechanistically, using both frog embryos and human iPSC cells, we found that Prdm12 acts in nociceptor specification together with the Ngn1/2 proneural factors by promoting TrkA expression, a receptor crucial for nociceptor survival and maturation. We also highlight the repression of the visceral sensory neuron master regulator Phox2b as an alternative mechanism by which Prdm12 promotes nociceptive fate in cranial sensory ganglia. Together, our results identify Prdm12 as an evolutionarily conserved essential regulator of nociceptor development, acting at different step of their development and controlling their specification via two distinct mechanisms

[35] A role for the tRNA-modifying enzyme Elp3 in tuft cell differentiation and in the immune response in the intestine

Caroline WATHIEU1,2,4,*, Sylvia TIELENS1,2,4,*, Marion Rolot6,7, Kateryna Shostak1,2,4, Pierre Close1,3,4,5, Benjamin Dewals6,7* and Alain CHARIOT 1,2,4,5*. *Equal contributions.
Université de Liège
Presenting author: Caroline Wathieu

The role of tRNA modifications in cancer development as well as in the immune response only start to be elucidated. Elp3 is the catalytic subunit of Elongator which promotes the mcm5s2 chemical modification of some tRNAs necessary for efficient protein translation. We previously showed that Elp3 is required in Wnt-driven tumor initiation, at least through the mRNA translation of Sox9 whose expression is essential for the maintenance of Lgr5+/Dclk1+/Sox9+ cancer stem cells (1). However, Elp3 is dispensable in intestinal homeostasis as the architecture of intestinal crypts is unaltered without Elp3. Yet, the number of tuft cells dramatically decrease when Elp3 is genetically inactivated, suggesting that some tRNA modifications specifically promote tuft cell differentiation. In order to better investigate the role of Elp3 in tuft cell differentiation and in the immune response in the intestine, we use a model in which tuft cells are expanded upon Nippostrongylus Brasiliensis mice infection. Upon genetic inactivation of Elp3 in the intestinal epithelium, tuft cell amplification, IL-25 production, ILC2 cell number and goblet cell expansion

are impaired and N. Brasiliensis expulsion is consequently delayed. Therefore, tRNA modifications play a key role in the immune response to parasite infection in the intestine. We will provide the first insights into molecular mechanisms underlying the role of tRNA-modifying enzymes in tuft cell differentiation. Reference 1. Ladang et al., The Journal of Experimental Medicine (2015), Nov 16;212(12):2057-75.

[36] Role of lysosomal cysteine proteases in human bone marrow derived mesenchymal stromal cells differentiation into hepatic-like cells

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Presenting author: Mian Long Xaymontry

Bone marrow derived mesenchymal stromal cells are able to differentiate into various cells, such as adipocytes, osteoblasts and hepatocyte-like cells. We are investigating a putative role of lysosomal cysteine proteases in the hepatogenic program of differentiation of these mesenchymal cells. We found an increase in cathepsins B and K expression during the initiation step of this differentiation program, at both the mRNA and the protein levels. The active forms of these enzymes were not detected in the culture medium, suggesting that they may act intracellularly, probably inside lysosomes. Concomitantly, we observed a striking drop in collagen type I abundance in cell lysates that follows a marked decrease in COL1A1 and COL4A1 mRNAs. We therefore hypothesize that the proteolytic activity of cysteine proteases could contribute to the decrease in collagen content during the early step of hepatogenic differentiation. Together with the reduced expression of collagen transcript, this could lead to the remodeling of extracellular matrix before the maturation step. Investigations are currently ongoing to both identify the signaling pathways responsible for these modifications and determine whether cysteine cathepsin activation and collagen decrease are crucial for the differentiation of mesenchymal stromal cells into hepatocyte-like cells.