27th Edition

Pharmacology Research Day

May 11 2023

McGill | Department of Pharmacology and Therapeutics

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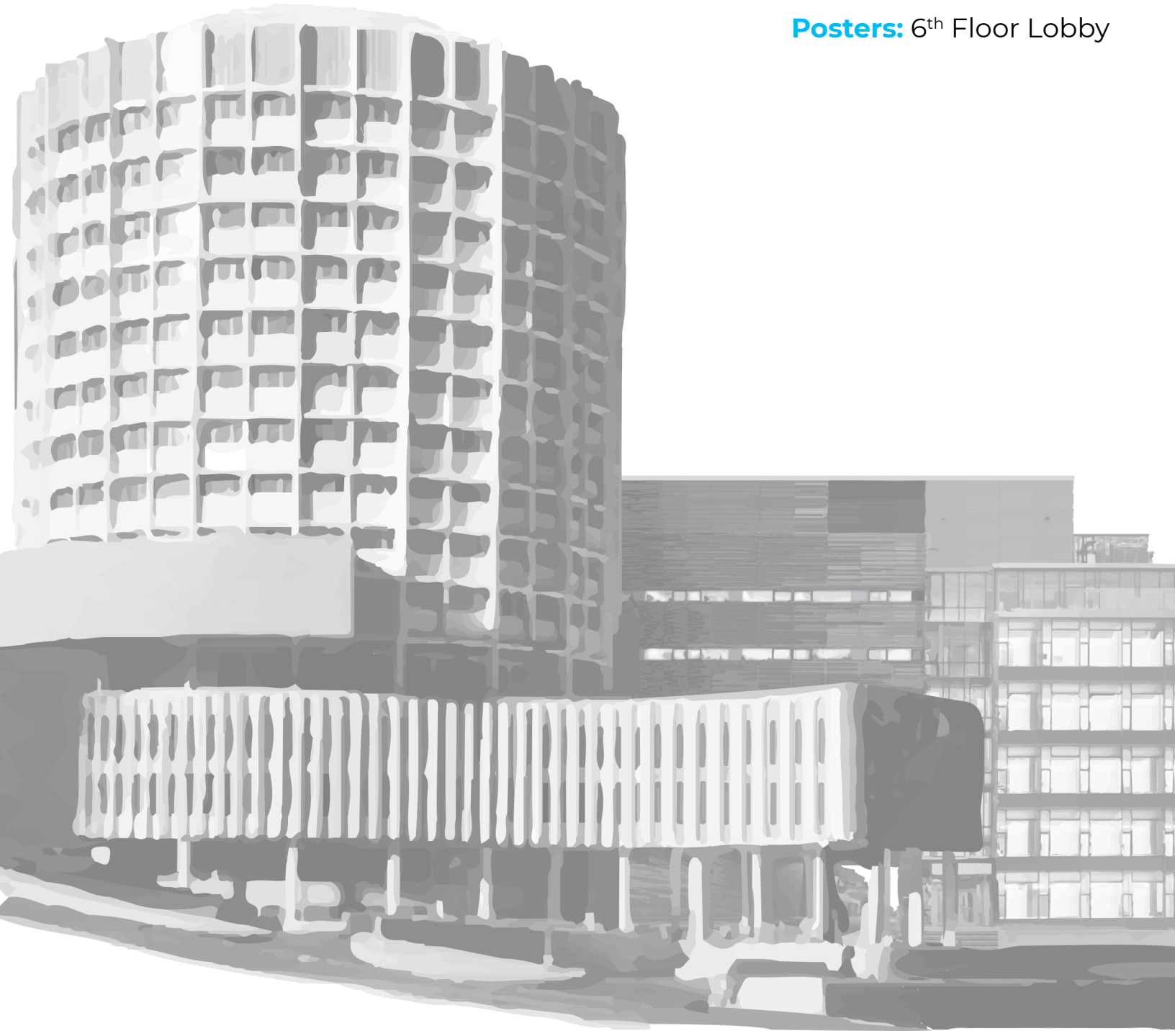
Location

McIntyre Medical Building

3655 Promenade Sir William Osler, Montreal, H3G 1Y6

Talks: Palmer Amphitheatre (Room 522)

Posters: 6th Floor Lobby



PRD Committee

Name	Position
Ajitha Thanabalasuriar	Co-Chair
John Walter Gillard	Co-Chair
Tina Tremblay	GPC and Administrative Assistant for the Committee
Adithi Sundarakrishnan	Student Member
Bruktawit Maru	Student Member
Deanna Sosnowski	Student Member
Hanan Mohammad	Student Member
Jacob Blaney	Student Member
Jasmine Phénix	Student Member
Negin Ghandhariyoun	Student Member
Rebecca Cummer	Student Member
Reilly Pidgeon	Student Member

Foreword

Dear colleagues,

We are excited to finally welcome everyone to the 27th edition of Pharmacology Research Day (PRD) held in the McIntyre Medical Building. We are eager to hear about the progress made by students of the Department of Pharmacology and Therapeutics and its associated departments since last spring.

We would also like to welcome our keynote speaker: **Dr. Christine Allen, PhD** from the University of Toronto and adMare Bioinnovations. We look forward to hearing about her ongoing research in the field of drug development through her talk entitled *Data Driven Drug Formulation and Development*.

We would like to extend special thanks to **event sponsors** for their generous contributions, supporting staff (Tina Tremblay, Chantal Grignon, Cathy Shang Kuan, and Hong Qin) who worked behind the scenes to make this event possible, and our co-chairs, Dr. Ajitha Thanabalasuriar and Dr. John Walter Gillard, for their dedication and hard work in planning this event.

Sincerely,

Students of the 27th Pharmacology Research Day Committee

Left to Right: Rebecca Cummer, Reilly Pidgeon, Deanna Sosnowski, Hanan Mohammad, Adithi Sundarakrishnan, Bruktawit Maru, Jacob Blaney

Absent: Jasmine Phénix, Negin Ghandhariyoun

Program

8h00-8h45	**Registration** - *6th Floor Lobby, McIntyre*
8h45	**Pharmacology Research Day Introduction** - *Palmer Amphitheatre, Room 522* - Dr. Terry Hébert, Graduate Program Director
9h00-10h00	**Keynote Address** - *Palmer Amphitheatre, Room 522* *"Data Driven Drug Formulation and Development"* Professor Christine Allen, PhD Leslie Dan Faculty of Pharmacy Department of Drug Development and Disease Diagnostics
10h00-11h30	**Break & Poster Session I** - *6th Floor Lobby, McIntyre* Odd Numbered Posters
11h30-13h00	**Oral Session I** - *Palmer Amphitheatre, Room 522*
11h30	Rebecca Cummer - PhD Student (Castagner Lab)
11h45	Irem Ulku - Postdoc (Multhaup Lab)
12h00	Quazi Islam - PhD Student (Baglole Lab)
12h15	Evan Buddle - MSc Student (Bernard Lab)
12h30	Ylauna Penalva - IPN PhD Student (Munter Lab)
13h00-14h00	***Lunch*** - *5th Floor Cafeteria* (Pick Up Lunch on *6th Floor*) **Lunch and Learn** with Nicolas Audet and Mark Hancock, *Room 522* (Prizes from Diamed to be won!)
14h00-15h30	**Poster Session II** - *6th Floor Lobby, McIntyre* Even Numbered Posters
15h30-16h15	**Oral Session II** - *Palmer Amphitheatre, Room 522*
15h30	Mozhdeh Mehdizadeh - PhD Candidate (Nattel Lab)
15h45	Joshua Emmerson - PhD Candidate (Cuello Lab)
16h00	Andrew Bayne - PhD Student (Trempe Lab)
16h15-17h00	**3-Minute Flash Talks** - *Palmer Amphitheatre, Room 522*
	Jamie Mustian - PhD Student (McKinney Lab) Simran Dhir - MSc Student (Ribeiro-da-Silva Lab) Elaine Xing - MSc Student (Castagner Lab) Adithi Sundarakrishnan - PhD Student (Clarke Lab) Tara Shomali - PhD Student (Trempe Lab) Grace Mazarura - Msc Student (Hébert Lab) Mary Loka - IPN PhD Student (Bernard Lab) Braeden Giles - PhD Student (Robaire/Mann Labs) Jacob Blaney - PhD Student (Hébert/Tanny Labs)
17h00-18h00	**Awards/Closing Remarks** - *6th Floor Lobby, McIntyre* (Also, Wine and Beer)

Keynote Speaker

Christine Allen, PhD

Full Professor
Leslie Dan Faculty of Pharmacy
University of Toronto

Vice-President
Ecosystems Development
adMare Bioinnovations

Dr. Allen is a Full Professor in the Leslie Dan Faculty of Pharmacy at the University of Toronto (U of T) where she has had a stellar 20-year career as researcher and academic leader. During her time at U of T, she held increasingly senior leadership roles, most recently as inaugural Associate Vice-President and Vice-Provost, Strategic Initiatives.

Dr. Christine Allen is the inaugural Vice-President, Ecosystem Development at adMare Bioinnovations. She is a world-renowned and award-winning researcher, experienced leader and entrepreneur.

In her role as Vice-President, Ecosystem Development at adMare Bioinnovations, Dr. Allen is continuing adMare's leadership in building a robust ecosystem for Canada's life sciences industry through providing state-of-the-art infrastructure, scientific and commercial expertise, and investment capital to companies, enabling them to grow and maintain operations in Canada. She also oversees the adMare Academy, building the talent that the Canadian ecosystem needs to drive the growth of the life sciences sector.

Dr. Allen is a globally renowned expert in drug formulation and development with more than 150 publications to her name and numerous successful collaborations with clinical and industry partners. She is co-founder and former president of Nanovista Inc., a company focused on high-precision, image-guided cancer therapy (featured by Scientific American as one of 10 start-ups changing healthcare).

Dr. Allen is a fellow of the American Institute for Medical and Biological Engineering, incoming President of the Controlled Release Society (CRS), and past president of the Canadian Society of Pharmaceutical Sciences (CSPS). She is Editor-in-Chief of the Journal of Controlled Release, a top ranked journal in the pharmaceutical sciences field.

Sponsors

Awards

Oral Presentation Award | Award for the best oral presentation

PhD Poster Award | Award for the best poster presentation by a PhD student in the Department of Pharmacology and Therapeutics

MSc Poster Award | Award for the best poster presentation by a MSc student in the Department of Pharmacology and Therapeutics

3-Minute Flash Talk Award | Audience choice award **(click me to vote!)** for the best 3-minute flash talk

Postdoc/RA/Tech Award | Award for the best presentation (oral or poster) by a postdoc/RA/Technician

Life Science Graduate Award | Award for the best poster presentation by a student supervised by a professor in the Department of Pharmacology and Therapeutics, but whose primary affiliation is not with Pharmacology

Judges

Name	Position
Nathan Luedtke	Faculty
Terence Edgar Hebert	Faculty
Ajitha Thanabalasuriar	Faculty
Bastien Castagner	Faculty
Ali Nejatie	Postdoc
Alfredo Ribeiro Da Silva	Faculty
Maureen McKeague	Faculty
Carolyn Baglole	Faculty
Paul Clarke	Faculty
John Gillard	Faculty
Jean-Sébastien Joyal	Faculty
Kalai Mangai Muthukumarasamy	Postdoc
Jason Tanny	Faculty
Daniel Bernard	Faculty
Dusica Maysinger	Faculty
Anne McKinney	Faculty
Sonia Do Carmo	Staff
Dominic Devost	Staff
Luisina Ongaro Gambino	Staff
Mark Hancock	Staff
Nicolas Audet	Staff

Oral Presentations

Format

The oral presentation is intended to give graduate students the opportunity to discuss multiple aspects of their research project. It is also an opportunity to receive criticism and feedback from peers and faculty members on their current work.

Duration: 10 minutes

Question period: 5 minutes

Schedule

11h30-13h00 **Oral Session I** - *Palmer Amphitheatre, Room 522*

11h30	Rebecca Cummer - PhD Student (Castagner Lab)
11h45	Irem Ulku - Postdoc (Multhaup Lab)
12h00	Quazi Islam - PhD Student (Baglole Lab)
12h15	Evan Buddle - MSc Student (Bernard Lab)
12h30	Ylauna Penalva - IPN PhD Student (Munter Lab)

15h30-16h15 **Oral Session II** - *Palmer Amphitheatre, Room 522*

15h30	Mozhdeh Mehdizadeh - PhD Candidate (Nattel Lab)
15h45	Joshua Emmerson - PhD Candidate (Cuello Lab)
16h00	Andrew Bayne - PhD Student (Trempe Lab)

Synthesis and *in vitro* evaluation of IT3S3 as a therapeutic against *Clostridioides difficile* infection

Rebecca Cummer, Raphaël Bolteau, Lauren Finn, Bettina Keller, Bastien Castagner

Purpose: *Clostridioides difficile* (C. diff) is a bacterium that causes the most prevalent form of hospital-acquired infection. C. diff infection (CDI) is caused by antibiotics as they disrupt the gut microbiota that prevents C. diff from colonizing, resulting in high rates of disease relapse. Consequently, there is a need for new treatments against CDI. One treatment strategy would be to target toxins released by C. diff. The toxins make an ideal drug target as they are the primary cause of symptoms.

Method: We previously published an approach to target the toxins using analogues of inositol hexakisphosphate (IP6). IP6 is a natural co-factor of the toxin's cysteine protease domain (CPD), responsible for triggering the auto-processing of C. diff toxins in enterocytes. Our analogues pre-emptively induce toxin cleavage, neutralizing them before they enter enterocytes. We have synthesized new IP6 analogues to further explore their structure-activity-relationship with the toxin. First, we determined the ability of the IP6 analogues to cleave the holotoxin in vitro. Second, we used isothermal calorimetry to quantify the dissociation constants of the compounds with the CPD. Next, we performed a thermal shift assay to determine the stability of the toxin/analogue complexes. Finally, docking studies were performed to explore their binding pose in the CPD.

Results: A new IP6 analogue, IT3S3, significantly improved holotoxin cleavage compared to IP6 ($p \leq 0.01$). IT3S3 had a decreased dissociation constant ($K_{d,IP6}$ = 1.36µM, $K_{d,IT3S3}$ = 500nM) and improved CPD stability compared to IP6 ($TM_{,IP6}$ = 60.8ºC, $TM_{,IT3S3}$ = 63.6ºC). Finally, IT3S3 and IP6 maintained alternative binding poses in the CPD.

Conclusion: IT3S3's decreased dissociation constant improved its ability to cleave holotoxin in comparison with IP6. This was not observed with our previous lead compound, IT2S4. By enhancing toxin cleavage, we anticipate IT3S3 will outperform IT2S4 when tested in a fulminant mouse CDI model.

Acknowledgements | Funding: Canadian Institutes of Health Research (CIHR), Centre de Recherche en Biologie Structurale (CRBS).

Enzymes Involved in Aβ clearance and Their Role in Aβ34 Degradation

Irem Ulku, Adeola Shobo, Gerhard Multhaup

1. Objectives: Alzheimer Disease (AD) is a chronic neurodegenerative disorder that is clinically characterized by a progressive decline in cognitive functioning. Neuropathologically, the hallmarks include brain atrophy, neurofibrillary tangles and senile plaques consisting of amyloid-β (Aβ) peptides. Recent research has revealed that Aβ clearance is equally important as its production in affecting AD progression.

Aβ production involves two enzymes, β-secretase (BACE1) and γ-secretase, resulting in the production of Aβ peptides of various lengths including aggregation-prone and toxic Aβ42. Our studies revealed that BACE1 can cleave longer forms of Aβ, like Aβ42, and convert such forms into a non-toxic and non-aggregating Aβ34. However, not much is known about the metabolism of Aβ34 which is supposed to serve as an important biomarker of amyloid clearance in CSF. Therefore, we investigated the enzymes responsible for Aβ34 degradation.

2. Methods: We used a combination of three approaches: siRNA knockdowns, transient overexpressions and pharmacological inhibitions of candidate proteases in both wild-type and BACE1-overexpressing neuroblastoma cells. Expression levels of proteases were verified by Western blot and Aβ species were quantified by ELISA.

3. Results: Our studies identified several proteases within the endo-lysosomal system as prime candidates for Aβ34 degradation where Aβ34 is also produced by BACE1. Here, we provide evidence that Endothelin-Converting Enzyme-1 is the enzyme that is not only predominantly responsible for producing active Endothelin-1 but also possesses a major role in Aβ34 degradation.

4. Conclusions: A fundamental understanding of the metabolism of prognostic markers is critical and urgently needed for their further development as AD biomarkers, e.g., Aβ34 and the enzymes involved in the production and degradation of Aβ species. Therefore, our recent discoveries will pave the way for the development of novel tools used for early diagnosis of AD.

A multi-omics approach identifies that human antigen R (HuR) controls multiple cellular pathways in idiopathic pulmonary fibrosis (IPF) pathogenesis

Quazi S. Islam[1,5], Steven K. Huang[3], David H. Eidelman[1,2], Carolyn J. Baglole[1,2,4,5]

[1]Research Institute of the McGill University Health Centre, Montreal, Canada, [2]Department of Medicine, McGill University, Montreal, Canada, [3]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA, [4]Department of Pathology, McGill University, Montreal, Quebec, Canada, [5]Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada

Idiopathic pulmonary fibrosis (IPF), a chronic irreversible disease, is characterized by permanent scarring of the lung that results in death from respiratory failure. A key driver of this scarring is the accumulation of myofibroblasts, induced by transforming growth factor β (TGF-β), producing excessive extracellular matrix (ECM) proteins. Recently, we showed that human antigen R (HuR) drives the differentiation of normal lung fibroblasts (NLFs) into myofibroblasts in response to TGF-β along with ECM protein production. HuR is an RNA-binding protein that controls numerous cellular functions including differentiation, survival, and proliferation. We hypothesized, dysregulated function of HuR in IPF lung fibroblasts (IPF-LFs) drives disease pathogenesis through numerous cellular pathways. NLFs and IPF-LFs were obtained from the University of Michigan Interstitial Lung Disease Biorepository and cultured under standard conditions. Fibroblasts were treated with TGF-β (5 ng/ml) following HuR knockdown with siRNA. Then, mass spectrometry RNA-sequencing (RNA-Seq) and (MS)-based proteomics were performed to evaluate the changes in HuR-regulated RNA and protein, respectively. RNA-Seq revealed that HuR controlled numerous genes involved in pathways associated with ECM organization and response to wounding pathways in IPF-LFs. Proteomic analysis revealed that in both NLFs and IPF-LFs, HuR controls the expression of numerous proteins associated with IPF pathogenesis, including those involved in the ECM (COL1A1), cell survival (IFGBP2), and protein translation (EIF4) in response to TGF-β. Pathway analysis revealed that HuR is essential for the expression of proteins involved in ECM organization, response to wounding, etc. in IPF-LFs. This study provides novel information about many established and novel pathways being regulated by HuR in lung fibroblasts under myofibroblast differentiating conditions. Several pathways are controlled by HuR only in IPF-LFs, suggesting that there is a dysfunction of HuR in IPF.

Porcine TGFBR3L functions as an inhibin B co-receptor *in vitro*

Evan R. S. Buddle[1], Gustavo Zamberlam[2], Vilceu Bordignon[3], Daniel J. Bernard[1,4]

[1]Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada, [2]Département de Biomédecine Vétérinaire, Université de Montréal, Montreal, Quebec, Canada, [3]Department of Animal Science, McGill University, Montreal, Quebec, Canada, [4]Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada

Follicle-stimulating hormone (FSH) is released from pituitary gonadotrope cells and promotes ovarian follicle development in females. FSH production is stimulated by activin-class transforming growth factor β (TGFβ) ligands, which act via complexes of activin type II and I receptor serine/threonine kinases. Inhibins suppress FSH by competitively binding to and sequestering type II activin receptors. Inhibins bind these receptors avidly in the presence of co-receptors. Two inhibin co-receptors have been described, TGFβ type III receptor (TGFBR3 or betaglycan) and TGFBR3-like (TGFBR3L). Betaglycan mediates the actions of both inhibin A and B, and is ubiquitously expressed. In contrast, TGFBR3L is restricted to gonadotropes and is an inhibin B-specific co-receptor. *Tgfbr3l* knockout mice exhibit elevated FSH levels and enhanced fertility, due to reduced inhibin B action in gonadotropes. To determine whether TGFBR3L may similarly mediate inhibin B action in other species, we cloned and characterized the *Tgfbr3l* cDNA from porcine pituitary. When expressed in heterologous cells, porcine TGFBR3L was expressed at the plasma membrane and exhibited a glycosylation pattern similar to that observed with murine and human orthologs. Porcine TGFBR3L conferred inhibin B, but not inhibin A sensitivity in a heterologous promoter-reporter assay. Our results suggest that porcine TGFBR3L functions as an inhibin B specific co-receptor *in vitro*.

Acknowledgements | Funding Sources: Supported by NSERC USRA & CGSM, FRQNT.

Knockout of an ER proteostasis regulator rescues cognition in Alzheimer's disease model

Ylauna Penalva, Sandra Paschkowsky, Jingyun Yang, Francois Charron, Anne McKinney, David A. Bennett and Lisa Munter

Background: Characteristic cerebral pathophysiological changes of Alzheimer's disease (AD) such as glucose hypometabolism or the accumulation of cleavage products of the amyloid precursor protein (APP), known as Aβ peptides, lead to sustained ER stress and neurodegeneration. To preserve ER homeostasis, cells activate their unfolded protein response (UPR). The rhomboid-like-protease 4 (RHBDL4) is an enzyme which participates in the UPR by targeting ubiquitinated proteins for proteasomal degradation. We demonstrated that RHBLD4 cleaves APP in HEK293T cells, leading to decreased total APP and Aβ. We confirmed this processing pathway in ex vivo mixed cortical murine cultures. We aim to determine the physiological relevance of RHBDL4 in AD.

Methods: RHBDL4 mRNA and protein expression was assessed in AD patients as well as in an AD mouse model expressing human APP (J20). We then crossed J20 mice to a RHBDL4 knockout (KO) model to determine the effects of RHBDL4's absence on APP expression and Aβ in brain samples. We assessed cognition using the Y maze and the NOR test. Signaling pathways involved in cognition were studied in mouse samples and RHBDL4 KO MEFs.

Results: Both human and J20 brain samples have increased RHBDL4 mRNA and protein expression. RHBDL4 knockout in J20 mice leads to increased total cerebral APP and Aβ levels when compared to J20 alone. However, our tests showed that RHBDL4 absence rescued cognition in 5 months old female J20 mice, while males were not impaired yet. Interestingly, we demonstrated in vitro and in vivo that RHBDL4 absence leads to greater levels of active β-catenin due to decreased proteasomal activation. Therefore, we propose that the absence of RHDBL4 increases β-catenin signaling, whose decreased activity has been published to underlie cognitive defects in J20 mice and AD.

Conclusion: Our work suggests that RHBDL4's increased expression in AD, likely to decrease APP and Aβ levels, leads to aberrant degradation of β-catenin.

Accumulation of Cardiac Senescent Cells leads to Age-related Cardiac Remodeling in Mice

Mozhdeh Mehdizadeh, Martin Mackasey, Patrice Naud, Allan Ochs, Kimia Gharagozloo, Nhung Vuong, Eric Thorin, Gerardo Ferbeyre, Jean-Claude Tardif, Martin G. Sirois, Stanley Nattel

Background: Age is the leading risk factor for cardiac disease. However, how aging leads to cardiac disease is unknown. Senescence is an aging and stress related response. The role of senescence in age-related cardiac remodeling is unknown.

Objectives: To study the effect of clearance of senescent cells (SCs) on cardiac remodeling in aging mice.

Methods: We used INK ATTAC mice, a transgenic mouse model in which senescent cells undergo apoptosis following treatment with an agent, AP20187 (AP). Mice were treated with AP or vehicle from 12 months to 18 months. Cardiac function and structure were studied with echocardiography at baseline and end-study. LV pressure and isovolumic relaxation time were measured with a Millar catheter at the end-study. The percentage of SCs in different cardiac cells was analyzed with Fluorescence-Activated Cell Sorting (FACS) or immunofluorescence.

Results: From 12 to 18 months, LV mass/LV diameter at end diastole (LVDd) and the LV anterior wall thickness at end diastole (LVAWd) increased in the vehicle group while they were normalized with AP (LV mass/LVDd: Δvehicle=+2.6± 0.7, ΔAP=+0.6+0.4, P=0.027; LVAWd: Δvehicle=+0.05±0.02 AP=-0.005 ±0.01P=0.012). Isovolumic relaxation time indicated diastolic dysfunction in vehicle mice and improved with AP. Analysis of green fluorescent protein (GFP), indicating SCs, with FACS, showed that the number of senescent fibroblasts decreased with AP, while immune and endothelial cells were not affected. Also, immunofluorescence data showed senescent cardiomyocytes were reduced with

AP, suggesting that in our model improvement in age-related cardiac remodeling might be mediated through the clearance of senescent fibroblasts and cardiomyocytes.

Conclusion: Our study points to a significant role of SCs, likely senescent fibroblasts, and cardiomyocytes, in age-related cardiac remodeling. Modulating senescence might be a novel therapeutic approach for age-related diseases.

Acknowledgements | CIHR, FRQS

Neurovascular compromise and neurodegeneration in a transgenic rat model of human tauopathy with higher translational value

Joshua T Emmerson, Ai Liu, Quentin Bonomo, Sonia Do Carmo, and A. Claudio Cuello

Tauopathies, including frontotemporal dementia (FTD) and Alzheimer's disease (AD), clinically present with progressive cognitive decline and the deposition of neurofibrillary tangles (NFTs) in the brain. Neurovascular compromise is also prevalent in AD and FTD however the relationship between tau and the neurovascular unit is less understood relative to other degenerative phenotypes. Current animal models confer the ability to recapitulate aspects of the CNS tauopathies, however, existing models either display overaggressive phenotypes, or do not develop neuronal loss or genuine neurofibrillary lesions.

In this report, we communicate the longitudinal characterization of brain tauopathy in a novel transgenic rat model, coded McGill-R955-hTau. This transgenic rat expresses the longest isoform of human P301S tau. Homozygous R955-hTau rats displayed a robust, progressive accumulation of mutated human tau leading to the detection of tau hyperphosphorylation and cognitive deficits accelerating from 14 months of age. This model features extensive tau hyperphosphorylation with endogenous tau recruitment, authentic neurofibrillary lesions, and tau-associated neuronal loss, ventricular dilation, decreased brain volume, and gliosis in aged rats. Further, we demonstrate how neurovascular integrity becomes compromised at aged life stages using a combination of electron microscopy, injection of the tracer horseradish peroxidase and immunohistochemical approaches.

Acknowledgements | FRQS Doctoral training fellowship to JTE; CIHR, Canadian Consortium of Neurodegeneration and Aging (CCNA), Healthy Brains Healthy Lives and BrainsCAN McGill Western Collaboration grant (MWCG), Morris and Rosalind Goodman Family Foundation to ACC's laboratory.

Characterization of the PINK-TOM complex: a key target in Parkinson's disease

Andrew N. Bayne, Mohamed Eldeeb, Edward A Fon, Jean-Francois Trempe

Mitochondrial quality control is central to the etiology of Parkinson's disease (PD). Specifically, mutations in Parkin and PINK1, two proteins which coordinate the selective removal of damaged mitochondria (coined "mitophagy") are known to cause early-onset autosomal PD. PINK1 is a mitochondrially targeted kinase that accumulates on the outer mitochondrial membrane (OMM) following various mitochondrial stressors, where it dimerizes, phosphorylates ubiquitin, and activates Parkin, an E3 ligase that drives mitophagy. The accumulation of PINK1 on the OMM is critical for its function, and PINK1 accumulation hinges on its interaction with the TOM complex – the essential translocase that imports mitochondrial proteins and also acts as a signalling hub for translation, folding, immune signalling, and more. While it has been shown that accumulated PINK1 forms a stable, high-molecular weight complex with specific components of the TOM machinery, very little is known about how PINK1 specifically interacts with certain subunits, and which other interactors might bind to PINK1 and stabilize its TOM-bound form. In this work, we set out to characterize key determinants for PINK-TOM complex formation through a variety of structural and biochemical techniques. We utilize structural models and in vitro validation to identify critical motifs within PINK1 that bind to specific TOM subunits and are required for downstream PINK1 signalling. We also highlight key PD-linked mutations within these motifs which may disrupt PINK-TOM complex formation. Finally, we employ an unbiased mass spectrometry-based approach to identify novel components of the PINK-TOM complex in order to shed light on which other uncharacterized mitochondrial proteins are critical for the PINK1 signalling cascade. Overall, our work provides biochemical insight into the mechanisms which drive PINK-TOM formation and provide rationale for how disruption of this complex may lead to PD.

Acknowledgements | CIHR, Michael J Fox Foundation

3-Minute Flash Talks

Format

The 3-Minute Flash Talk presentation is intended to give graduate students the opportunity to give a brief overview of their research project. The audience are members of the Department of Pharmacology, therefore the language used can be more specialized, though field-specific jargon should be minimized.

Duration: 3 minutes

Question period: N/A.

Schedule

16h15-17h00 **3-Minute Flash Talks** - *Palmer Amphitheatre, Room 522*

Jamie Mustian - PhD Student (McKinney Lab)
Simran Dhir - MSc Student (Ribeiro-da-Silva Lab)
Elaine Xing - MSc Student (Castagner Lab)
Adithi Sundarakrishnan - PhD Student (Clarke Lab)
Tara Shomali - PhD Student (Trempe Lab)
Grace Mazarura - Msc Student (Hébert Lab)
Mary Loka - IPN PhD Student (Bernard Lab)
Braeden Giles - PhD Student (Robaire/Mann Labs)
Jacob Blaney - PhD Student (Hébert/Tanny Labs)

Audience Choice Award

Scan me to vote!

(or click the link below)

Jamie Mustian Pharmacology | PhD | F1

Mistrafficking of KCC2 in Endolysosomal system in the hippocampus of Christianson Syndrome mouse model

Mustian, J., Gao, AYL., Shi, R., Orlowski, J., and McKinney, RA

Christianson Syndrome (CS) is an X-linked neurodevelopmental/neurodegenerative disorder. Patients have intellectual disability, autism and epilepsy. It arises from mutations in the SLC9A6 gene which normally encodes the endosomal pH regulator (Na+, K+)/H+ exchanger isoform 6 (NHE6). NHE6 maintains the accurate pH of endosomes to allow proper cargo trafficking. All mutations result in a loss-of-function for NHE6 and an overacidification of recycling endosomes is observed. Epilepsy and autism have been linked to low levels of 2 K+/Cl− cotransporter (KCC2). As KCC2 is located in recycling endosomes we investigated if loss of NHE6 resulted in low levels and or mistrafficking of KCC2 to overacidification of endosomes which could lead to autism and epilepsy in CS patients. Using immunoblotting in a murine model of CS (NHE6KO) we found that KCC2 levels were significantly lower in the hippocampi of KO vs WT at PND 21, 60, and 180. There were no significant differences in mRNA levels of KCC2 between KO and WT indicating gene transcription may not underlie the alterations in protein expression. Using immunolocalization we tested mislocalization of KCC2 in acute hippocampal slices of P60 tissue using LAMP1 (lysosomal marker) and Rab11(recycling endosome marker). We found a significant increase in colocalization of KCC2 with LAMP1 in the KO and less in recycling endosomes compared to WT suggesting mistrafficking of KCC2. The present study elucidated fundamental and CS disease-relevant mechanisms in KCC2 trafficking to potentially rescue the mislocalization of KCC2 in future studies.

Acknowledgements | CIHR and Graduate Excellence Fellowship

Contribution of structural synapse plasticity affecting dorsal horn projection neurons in the maintenance of neuropathic pain

Simran Dhir, Alfredo Ribeiro-da-Silva

Neuropathic pain (NP) is a chronic and ineffectively treated condition resulting from damage to the somatosensory nervous system. Spinal dorsal horn (DH) pathways involved in the coding of sensory information in NP have been proposed as pain relief targets. While evidence suggests that DH inhibitory transmission deficits lead to NP, directly targeting this disinhibition is challenging. Recently, we found that after nerve injury, microglia, through complement-dependent mechanisms, mediate the elimination of DH synapses, causing a net loss of inhibitory synapses. However, these studies did not evaluate synaptic inputs on specific neuronal populations. Here, we investigate the role of microglia and complement-mediated synapse pruning on synaptic inputs on lamina I (LI) projection neurons. As these neurons receive synapses from primary afferents, excitatory interneurons and inhibitory interneurons and transmit pain signals to the brain, changes in their synaptic inputs would directly affect the nociceptive output to the brain.

Projection neurons will first be identified by injecting Ai14 reporter mice in the lateral parabrachial nucleus with retro adeno-associated virus expressing Cre. Mice with spared nerve injury, modelling peripheral neuropathy, or sham surgery will be treated with vehicle, complement factor C1q neutralising antibodies or microglia inhibitor and tested at baseline and weekly for mechanical hypersensitivity, heat hyperalgesia, and cold allodynia. DH sections shall be stained for neuronal, microglia, and complement markers. Neuron activation, inhibitory and excitatory synapse densities per neuron, co-localization of C1q and synapse markers, and synapse engulfment will be compared.

This study highlights the synaptic plasticity in the DH circuit after nerve injury and provides a structural basis for the maintenance of NP. Further functional studies, including electrophysiology, will investigate how synapse density changes relate to neuronal function.

Acknowledgements | Funded by The CIHR and Louise and Alan Edwards Foundation. Thank you to the Ribeiro-da-Silva, Khoutorsky and Prager-Khoutorsky Lab members. This wouldn't have been possible without the support of my committee members, friends, and family.

Isolation of arabinoxylan consumers in the human gut microbiota using fluorescent glycan labeling

Elaine Xing, Bastien Castagner

The composition of the gut microbiota has important consequences in human health. A loss of biodiversity in gut bacteria, known as dysbiosis, is implicated in disease states including inflammatory bowel disease, obesity, and Clostridioides difficile infection. Balance in the endogenous bacterial composition can be restored using prebiotics, which are molecules such as glycans and polyphenols, capable of supporting specific bacteria. Diet-derived complex glycans are being investigated for their potential as prebiotics since gut bacteria use these carbohydrates as an energy source. The gut microbiome is enriched with genes encoding carbohydrate-active enzymes that are needed to break down complex glycans. Hence, we can shape the gut microbiota using diet. However, glycan metabolism in the gut microbiota is still not completely elucidated. Furthermore, clinical trials and animal studies have focused on only a few glycan structures for their potential as therapeutic prebiotics, such as fructooligosaccharide, despite the vast repertoire of diet-derived glycans that are metabolized by the gut microbiota. Arabinoxylan (AX), the main non-starch polysaccharide in wheat bran, is a prebiotic glycan that can be further investigated. Currently, we are using a workflow that metabolically labels stool samples with fluorescently-labelled glycans, and isolates labeled bacteria using FACS and culturomics. Positive glycan consumption phenotype was validated using growth curves and bacteria species were identified using DNA extraction and 16S rDNA sequencing of the entire 16S gene (V1-V9 region). Beyond optimizing this workflow for the isolation of AX consumers from the human gut microbiota, this work presents the potential of the metabolic labeling workflow to study glycan metabolism of lesser investigated glycans.

Ultrasonic vocalizations as a measure of depressive-like affect

Adithi Sundarakrishnan & Paul Clarke

Ultrasonic vocalizations are calls emitted by rodents at two main frequencies, 22 kHz and 50 kHz, that are beyond our hearing range. Of particular interest, rats make many different types of 50-kHz calls and some of these reflect their current emotional state. Measuring the affective state of a rodent can help us improve animal models of psychiatric disorders, which do not adequately capture symptoms seen in humans. This is evident in animal models of depression, specifically in the measurement of the core symptom of anhedonia. Anhedonia is often defined as an inability to derive pleasure from previously pleasurable stimuli and is measured as a decreased preference for sweet tasting water in animal models of depression. However, recent evidence suggests that depressed patients do not reliably show an analogous deficit. In fact, depressed individuals may fail to anticipate how much pleasure they will experience, without showing any deficits in experiencing pleasure from rewarding stimuli. Ultrasonic vocalizations can be harnessed in the study of affect in animal models of depression, as novel measures are needed. I will measure ultrasonic vocalizations in rats under a depressive-like state and look for variations in the emitted calls, which might reflect an anhedonia-like symptom. Using ultrasonic vocalizations may allow us to capture the emotional component of depression and thereby improve the mapping of human symptoms onto animals, which could ultimately aid in the development of more effective antidepressant therapies.

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Small molecule PINK1 modulators

Tara Shomali, Shafqat Rasool, Nathalie Croteau, Simon Veyron, Luc Truong, Jean-François Trempe

Parkinson's disease (PD) is a debilitating neurodegenerative disease characterized by tremors, dementia, bradykinesia for which there is no effective disease modifying treatments. Autosomal recessive juvenile PD which is characterized by the onset of PD at ages below 50, is known to be caused by loss-of-function mutations in PINK1, a kinase that initiates mitochondrial autophagy via ubiquitin and PARKIN phosphorylation. Mutations in PINK1 negatively impacts the mitochondrial quality control pathway and result in the accumulation of mitochondrial damage associated with disease pathology. Modulation of PINK1 through small molecules could help reinstate PINK1's function and provide an important research tool. However, there are no modulators of Homo sapiens (Hs)PINK1, and the inability to purify it recombinantly prevents the use of high throughput screening. Instead, the more stable insect orthologue of PINK1, Tribolium castaneum (Tc)PINK1 was used in a screen against a kinase inhibitor library. The screen identified PRT062607 as an inhibitor of TcPINK1 with an IC50 of 1.6 ± 1.2 uM. The crystal structure of TcPINK1 bound to PRT062607 revealed binding to the ATP binding site. Despite recent progress, differences between HsPINK1 and TcPINK1 are significant as some modulators of recombinantly expressed TcPINK1 were not found to elicit the same response in cell-based assays with HsPINK1. We have overcome this challenge by developing a humanized TcPINK1 construct, in which human-like mutations were introduced in the ATP binding domain. Furthermore, this novel construct has yielded crystals which could provide a structure revealing the binding pocket of HsPINK1. Our work provides the baseline for development of PRT062607 derivatives with higher potency, and the humanized TcPINK1 construct provides a basis for HsPINK1 specific modulators, a method which has not been explored before for PINK1.

Modelling cardiac fibrosis in a dish: G protein-coupled receptor signaling in hiPSC-derived cardiac fibroblasts of dilated cardiomyopathy patients

Grace R Mazarura, Kyla Bourque, Darlaine Pétrin, Karima Alim, Terence Hébert

Dilated cardiomyopathy (DCM) is characterized by pathological remodeling of heart tissue which can ultimately lead to heart failure. The adverse/pathological cardiac remodeling in heart failure and DCM is attributed to activated cardiac fibroblasts (CFs). The angiotensin II type-1-receptor (AT1R) and endothelin-1 receptors (ETR) have been shown to activate the ERK signaling pathway - a pathway that is significantly upregulated in the failing hearts of DCM patients and is known to modulate the activation state of fibroblasts and fibrosis. However, signaling of these GPCRs remains understudied in fibroblasts in comparison to cardiomyocytes. Our objective is to create tools to investigate the signaling of GPCRs implicated in fibrosis to gain a better understanding of how fibroblasts become activated. Here, we use human induced pluripotent stem cell-derived cardiac fibroblasts (hiPSC-CFs) to elucidate signaling events driving the fibrotic response. We have optimized and validated an established protocol for differentiating hiPSCs to hiPSC-CFs. Following this, we used FRET-based biosensors to track ERK1/2 signaling downstream of AT1R and ETR stimulation in hiPSC-CFs derived from both healthy subjects and DCM patients. We hope that having a better grasp on the mechanisms underlying cardiac fibrosis will lead to the discovery of better therapies for DCM.

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FSH does not directly regulate adipogenesis via the FSH receptor in mice

Mary Loka, Luisina Ongaro, and Daniel J. Bernard

Rising rates of obesity are a serious public health challenge. Post-menopausal women are at higher risk of developing obesity and associated comorbidities than premenopausal women, with hormonal changes, such as reduced estrogens, likely to be contributing factors. Menopause is also characterized by increases in pituitary-derived follicle-stimulating hormone (FSH). Recent data suggest that FSH may directly stimulate adipogenesis and block thermogenesis in adipocytes. An FSH-neutralizing antibody reportedly reduced fat mass and increased lean mass in ovariectomized (OVX) female mice, a rodent model of menopause. In these studies, it was not established whether FSH acted directly through its canonical receptor (FSHR) in adipocytes to mediate its effects. To assess FSH actions in vivo, we generated adipocyte-specific *Fshr* knockout mice (*Fshrfx*/fx;*Adipoq*-Cre, hereafter cKO). Ten-week-old cKO and control female mice were OVX and maintained on standard chow. Eight to 10 weeks later, we performed glucose tolerance and insulin tolerance tests, and determined body composition by EchoMRI. Cre-mediated recombination of the floxed *Fshr* locus in adipocytes was successful, but did not alter body weight, fat or lean mass, or glucose metabolism in cKO relative to control OVX mice. Thus, our results challenge the hypothesis that FSH acts through the classical FSHR in adipocytes in vivo.

Environmentally Relevant Mixtures of Organophosphate Esters Suppress THP-1 Macrophage Phagocytosis

Braeden Giles, Nikola Kukolj, Bernard Robaire, Koren Mann

Organophosphate esters (OPEs) are a class of toxic chemicals used as flame retardants and plasticizers in building materials and commercial goods. Some OPEs can harm the immune system; specifically, by disrupting the ability of macrophages to engulf and digest foreign particles and cells through phagocytosis. However, research thus far has focused on individual OPEs. In the environment, OPEs exist as mixtures in which 1) certain OPEs have an increased relative abundance; and 2) some OPEs have opposing activities. In the present study, we tested the hypothesis that a Canadian household dust-based OPE mixture will decrease phagocytic uptake of apoptotic cells and bacterial pathogens. To test our hypothesis, THP-1 macrophages were exposed to the OPE mixture for 48 hours and the resulting dose-response relationship was examined. We report that the OPE mixture dose-dependently suppresses efferocytosis, the engulfment of apoptotic cells. This decrease is associated with reduced expression of receptors for the apoptotic marker phosphatidylserine. Furthermore, the decreased engulfment of apoptotic cells is accompanied by a similar decrease in phagocytosis of gram-negative (Escherichia coli) and gram-positive (Staphylococcus aureus) bacteria. These findings suggest that mixtures of environmentally relevant OPEs can significantly impair the function of macrophages, which has important implications for human health and disease.

Acknowledgements | Supported by CIHR

Investigating the interaction between the SWI/SNF chromatin remodelling complex and Gβγ signalling in cardiac fibrosis

Jacob Blaney, Terry Hébert, and Jason Tanny

The formation of fibrotic tissue, mediated by cardiac fibroblasts, is an important repair and maintenance process in the healthy heart that becomes dysregulated during the pathogenesis of heart failure. Cardiac fibroblasts differentiate into myofibroblasts in response to cardiac damage, depositing extracellular matrix proteins and secreting fibrosis-inducing signalling molecules. Fibroblast stimulation by angiotensin II (Ang II) induces the fibrotic response by activating angiotensin II type 1 receptors (AT1R), a G protein-coupled receptor. We have found a non-canonical, nuclear function for Gβ subunits that regulates the fibrotic transcriptional program, and that implicates the BRG1 subunit of the SWI/SNF complex. We hypothesize that Gβ1 signalling acts as a transient break to suppress the fibrotic response through interactions with BRG1. To assess changes in the fibrotic response following siRNA knockdown of BRG1 or Gβ1 in rat neonatal cardiac fibroblasts (RNCFs), Ang II-induced proliferation, collagen secretion, and fibrotic gene expression have been compared between treatment conditions. Next, RNA sequencing and pathway analysis will be performed to survey the transcriptional control of the fibrotic response under Ang II and siRNA treatment conditions. Finally, gene loci where Gβ1/BRG1 interact will be identified using CUT&RUN. Our cultured RNCFs respond to Ang II agonism by increasing proliferation and expression of pro-fibrotic Serpine 1, however, collagen secretion is unaffected. In line with the proposed break mechanism, loss of Gβ1 increases Ang II-induced proliferation. We hope to understand why this braking mechanism seems to be selective using transcriptome and chromatin occupancy analyses, as well as further explore the transcriptional control of the fibrotic response.

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Poster Presentations

Format

The poster presentation is intended to give graduate students the opportunity to communicate their research in a concise and visually-engaging format. The poster presentation contains two components: a poster and a verbal presentation of the poster content.

Duration: 90 minutes/session

Question period: Conversational

Schedule

10h00-11h30 **Poster Session I**
Odd Numbered Posters

14h00-15h30 **Poster Session II**
Even Numbered Posters

Poster	Presenter	Poster	Presenter
1	Hanan Mohammad	**2**	Emma Paulus
3	Ali Shariat-Panahi	**4**	Jasmine Phenix
5	Sabrina Romanelli	**6**	Diego Loggia
7	Natali Joma	**8**	Stephanie Patterson
9	Deanna Sosnowski	**10**	Benjamin Kannel
11	Lucas Marques	**12**	Lama Iskandarani
13	Reilly Pidgeon	**14**	Ai Liu
15	Maddison Arlen	**16**	Vincenza Caruana
17	Emily Wilson	**18**	Ayse Zengin
19	Monika Kojic	**20**	Jérôme Bédard-Matteau
21	Andrew Little	**22**	Olivia Kovecses
23	Justin Meneses	**24**	Alyson Jiang
25	Xiaotong Wang	**26**	Pei You Wu
27	Dongwei Yu	**28**	Zixuan Li
29	Tapan Agnihotri	**30**	Robert Rizk
31	Yangfan Jin	**32**	Sofia Paoli
33	Bruktawit Maru	**34**	Ziyi Li
35	Patrick-Brian Bielawski	**36**	Nicole Heimbach
37	Rabah Dabouz	**38**	Seline Vancolen
39	Louis-Charles Masson	**40**	Garvit Bhatt
41	Hannah Derue		

Investigating the signalling and behavioural signatures of biased D1 receptor agonists in vivo

Hanan Mohammad, Jace Jones-Tabah, Terry Hébert, Paul Clarke

Despite considerable adverse consequences for patients, L-DOPA has remained the gold standard for the treatment of motor impairment in Parkinson's disease (PD) for the last 50 years. Remarkably, more efficacious drugs have not yet been developed. Dopamine D1 receptors (D1Rs) have been investigated for decades as a therapeutic target for motor impairment in PD. The search for clinically effective D1R agonists was, until recently, limited to drugs built on a catechol scaffold, resulting in poor pharmacokinetics and adverse cardiovascular effects. Recently, the discovery of the first non-catechol D1R agonists has revived clinical interest; these new compounds appear safe, well-tolerated and free of cardiovascular side effects. Non-catechol D1 agonists, as tested in vitro, showed greater potency in activating G protein-dependent signalling than in promoting β-arrestin recruitment, and were hence initially described as G protein-biased agonists. However, it is unknown whether these compounds show similar bias in vivo, and what the downstream functional consequences might be. Our labs have recently developed a novel technique combining virally-expressed biosensors with ratiometric fiber-photometry, to enable in vivo recording of GPCR signalling events in real-time in the rodent brain. We applied this approach to a 6-OHDA rat model of PD, to study how signalling that arises from D1R stimulation is conveyed through intracellular protein kinase A (PKA) and ERK1/2 activity. Here, we propose to utilize these and other in vivo biosensors to study the signalling and behavioural consequences of biased D1R signalling in a rat model of Parkinson's disease.

Acknowledgements | This project is supported by the Canadian Institutes of Health Research (CIHR)

Understanding G-protein and β-arrestin dependent signaling pathways at the Dopamine D1 Receptor

Emma Paulus, Hanan Mohammad, Terence E. Hebert, and Paul B.S. Clarke

When a ligand binds to a G-protein coupled receptor (GPCR), different signaling pathways can be modulated to transduce a signal. 'Biased ligands' preferentially stimulate or inhibit a subset of these signaling pathways. In the context of GPCRs, bias is often viewed as the distinction between G-protein-dependent and β-arrestin-dependent signaling. This has important therapeutic implications, as biased compounds could preferentially stimulate therapeutic pathways, thus providing fewer adverse effects. The therapeutic potential of biased agonists at the Dopamine D1 Receptor (D1R) for Parkinson's disease (PD) has largely been investigated. To date, many of these studies have been in heterologous systems, such as HEK 293 cells. We aim to continue the initial characterization of D1R ligand signatures in HEK 293 cells and further this study in rat neonatal primary striatal neurons (rPSN). Catechol and non-catechol D1R agonists are used to stimulate the D1R in HEK 293 cells and rPSNs. We hypothesize we will observe signaling bias for the non-catechol D1R compounds both between cell types (HEK 293 cells and rPSN), and within cell types due to intra-assay variability. In both systems, biosensors are used to detect either cAMP production, beta-arrestin recruitment, PKA or ERK 1/2 activation. For HEK 293 cells and rPSNs expressing FRET-based biosensors, high-content microscopy is used to image the cells and record biosensor signals following D1R agonist treatments. Whereas the catechol D1R agonists mostly display full agonism in both HEK 293 cells and rPSNs, the non-catechol agonists display either partial or full agonism depending on the signaling event (e.g., cAMP production or beta arrestin recruitment) and cell type. The differences in D1R agonists signaling profiles are likely due to system bias, rather than ligand bias alone. We will further assess system and ligand bias by determining the extent to which biased agonists show similar properties in the living brain.

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Functions of the C-terminal Repeat Domain of Spt5 in Transcriptional Regulation

Ali Shariat-Panahi, Jason C. Tanny

Regulators of transcription elongation by RNA polymerase II (Pol II) have emerged as potential novel therapeutic targets for diseases such as cancer, making it crucial to further understand their mechanism of action. Spt5 is an essential component of the Pol II elongation complex (EC) which directly interacts with the Rpb1 subunit of Pol II. Both Rpb1 and Spt5 have unstructured C-terminal domains (CTDs) composed of multiple amino acid repeats. The Rpb1-CTD directs co-transcriptional processes by recruiting other regulators and participates in the formation of molecular clusters through liquid-liquid phase separation (LLPS). The LLPS mechanism leads to accumulation of proteins in foci and is typically mediated by disordered protein domains. LLPS properties of the Rpb1-CTD and its interactions with other regulators are modulated by the phosphorylation state of CTD residues. Phosphorylation of Spt5-CTD is known to play important roles in Pol II transcription elongation. However, in contrast to the Rpb1-CTD, relatively little is known about its function. We propose that the Spt5-CTD has analogous functions to the Rpb1-CTD in transcription, including the formation of Spt5 droplets through LLPS and mediating the recruitment of EC components in a phosphorylation-dependent manner.

To better understand Spt5-CTD interactions with other regulators, we first performed affinity purification using phosphorylated and unphosphorylated Spt5-CTD peptides in the model eukaryote *Schizosaccharomyces pombe* and observed that both peptides yield protein bands on SDS-PAGE gels. Plasmids for introducing mRuby and mClover fluorescent tags to Spt5-CTD with 18 (wild-type) or 7 (truncated) repeats have also been constructed as the first step towards assessing the LLPS properties of the Spt5-CTD. The outcomes of these experiments will help in deciphering the roles played by Spt5 at the transcription elongation/termination stages and could provide novel opportunities for targeting human diseases.

Acknowledgements | This project is funded through CIHR grants to Dr. Jason Tanny.

The role of the cholesteryl ester transfer protein in Alzheimer's disease

Jasmine Phenix, Lisa Munter

High plasma cholesterol levels increase the risk of Alzheimer's disease (AD). The cholesteryl ester transfer protein (CETP), which increases cholesterol levels in low-density lipoproteins, has been associated with decreased AD risk. Thus, we hypothesize that CETP activity in an AD mouse model of amyloidosis will lead to cholesterol accumulation in the brain, reduced cholesterol excretion, increased production of amyloid-beta (Aß) peptides, and poor cognitive performance. Further, we propose that CETP inhibition will improve the symptoms of AD or prevent its pathology.

Wildtype (WT), McGill-Thy1-hAPP (hAPP), hCETPtg, and double-transgenic (hAPP/hCETPtg) mice fed a high cholesterol diet were injected daily i.p. with 30 mg/kg of evacetrapib or vehicle at 11-weeks of age for 10-weeks. Behaviour analyses and biochemical analyses were performed at 21-weeks of age.

As expected, hAPP and hAPP/hCETPtg mice showed cognitive impairment. Interestingly, evacetrapib rescued cognition in hAPP/hCETPtg mice. Good cognition correlated positively with higher HDL and lower LDL levels, but APP and Aß levels did not correlate with cognition. A ~25% cholesterol increase was found in hAPP mice brains and ~16% more cholesterol in hCETPtg mice on vehicle as compared to WT. Intriguingly, hAPP/hCETPtg mice on vehicle also only had ~16% higher cholesterol despite expressing APP indicating that CETP dominates the cholesterol load. Importantly, hCETPtg and hAPP/hCETPtg mice on evacetrapib had even higher cholesterol levels at ~38% above WT. Thus, CETP inhibition with evacetrapib increased cerebral cholesterol content against our hypothesis and was effective in rescuing cognitive performance.

We show that the relationship between brain cholesterol and cognition is more complex than a linear relationship; high brain cholesterol levels alone do not impair cognition. We expect that decreasing cholesterol dysfunction by drug repurposing CETP inhibitors will ameliorate AD pathology.

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The role of the Tom complex in the stabilization of PINK1 on damaged mitochondria

Sabrina Romanelli and Jean-François Trempe

Parkinson's disease (PD) is the most common movement disorder characterized by impairments, such as tremors and slowness of movement, and non-motor symptoms. By 2031, 163,000 Canadians will be diagnosed with PD, however current treatments do not slow the progression of the disease and have negative side effects. We are in dire need of new therapeutics. To do so, we must better understand the mutations related to PD. Mutations in Parkin and PINK1, two proteins involved in mitochondria quality control, have been known to cause early-onset PD. Parkin, an E3 ubiquitin ligase, removes mitochondrial damage by ubiquitinating outer mitochondrial membrane (OMM) substrates. Parkin is recruited to damaged mitochondria by PINK1, a mitochondrial kinase. Upon mitochondrial damage, PINK1 accumulates on the translocase of the outer membrane (TOM) complex, leading to its activation whereas in a healthy state PINK1 will be imported into the TOM complex and degraded. The TOM complex is a multimeric channel in the OMM important for protein transport. Although the PINK1-TOM complex is a key initiator of damage repair, it is unknown how PINK1 interacts with the TOM complex to prevent its degradation. Our goal is to better understand the role of the TOM complex in stabilizing PINK1 on damaged mitochondria. We will use genetically-encoded photocrosslinking and Blue-Native page to decipher key interactions forming the PINK1-TOM complex and will determine the structure of the proteins together. Increasing our understanding of the PINK1-TOM complex is crucial as it can be used as a potential new therapeutic target for PD.

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Novel role of citrate in human sperm capacitation

Diego Loggia and Cristian O'Flaherty

The prevalence of infertility is rising worldwide, with nearly half of these cases being traced to men. 30% of infertile men suffer from idiopathic infertility, thus there is currently an unmet need to characterize the mechanisms underlying male infertility.

To recognize and fertilize the oocyte, the spermatozoon must undergo a series of biochemical and morphological changes through the process of capacitation. For capacitation to occur, human spermatozoa require low ROS levels, activation of phosphorylation pathways, and sufficient energy metabolite levels.

Citrate is an abundant energy metabolite in seminal plasma, with low levels reported in idiopathic infertile men. In somatic cells, citrate can be exported from the mitochondrion to the cytosol via the mitochondrial citrate carrier (CIC). Citrate can then be used by the cytosolic ATP-citrate lyase (ACLY) to generate acetyl CoA and oxaloacetate, which can be used for intracellular nitric oxide (NO•) production. However, the specific role of citrate in sperm capacitation is unknown.

We hypothesize that citrate is required for energy and NO• production during sperm capacitation. We determined whether citrate, cytosolic ACLY, and mitochondrial CIC are involved in capacitation, and whether citrate promotes NO• production during capacitation.

Spermatozoa incubated with citrate and fetal cord serum ultrafiltrate (FCSu, a capacitation inducer) underwent capacitation at similar levels to spermatozoa incubated in BWW medium containing other energy substrates. In addition, ACLY and CIC inhibition prevented FCSu-induced capacitation in spermatozoa incubated in citrate-containing BWW medium. In these same conditions, L-NAME, an inhibitor of NO• synthesis, prevented capacitation.

These results demonstrate that cytosolic citrate supports sperm capacitation in a process involving NO• production. This research will help understand citrate regulation in sperm capacitation and will ameliorate treatment strategies for male infertility.

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Fisetin modulates oxidative stress in glioblastoma and the tumor microenvironment

Natali Joma, Issan Zhang, Laura McKay, Ashok Kakkar, Dusica Maysinger

Glioblastoma multiforme (GBM) is a highly aggressive brain cancer with significant therapeutic challenges. The current standard of care, temozolomide (TMZ), is widely ineffective due to increasing resistance. The tumor microenvironment (TME) has emerged as a valuable therapeutic target in cancer, as it promotes tumorigenesis through elevated production of reactive oxygen species (ROS). Non-neoplastic cells in the TME, such as microglia, accumulate near the hypoxic/necrotic core of the tumor, and in turn support tumor proliferation and angiogenesis. In this study, we investigated the potential therapeutic use of fisetin, a natural compound with antioxidative and anti-inflammatory properties, to modulate oxidative stress (OS) in microglia and GBM cells challenged with the viral stressor SARS-CoV-2 spike protein (SMT1-1). Our findings revealed that fisetin differentially modulates redox-responsive transcription factors (acHMGB1 and TFEB) in microglia and GBM. Specifically, fisetin decreased cytosolic acHMGB1 and increased TFEB abundance in microglia, but not in GBM. TFEB regulates the expression of genes necessary for lysosomal biogenesis and enzymatic activity. To test this model, we monitored lysosomal abundance and activity with membrane protein LAMP-2 and lysosomal protease cathepsin B, respectively. We found that fisetin increased lysosomal number and activity in microglia under OS. To enhance the pharmacokinetics of fisetin, we incorporated it into a polymeric micelle nano-formulation, which was found to not hinder the biological activity of fisetin. Overall, our results suggest that fisetin could normalize microglial activity disrupted in the TME by modulating OS. This effect on microglia could be beneficial in combination therapy, as it may enhance the effectiveness of primary therapy agents such as TMZ, and reduce putative side effects. Our findings also highlight the potential of using nanocarriers to improve the delivery of natural compounds in cancer therapy.

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Characterization of Phytocannabinoid Signaling Profiles at Cannabinoid Type 1 & Type 2 Receptor

Stephanie Patterson, Robert Laprairie, and Stephane Laporte

Cannabinoid type 1 (CB1R) and type 2 (CB2R) receptors have been implicated in many diseases. They were once believed to couple exclusively to the Gi/o protein family, but there is evidence they signal through alternate pathways. The aim of this project is to assess the signalling profiles and potential biases of eight phytocannabinoids at CB1R and CB2R: delta-9-tetrahydrocannabinol, cannabidiol, delta-9-tetrahydrocannabivarin, cannabidivarin, cannabigerol, cannabichromene, delta-9-tetrahydrocannabinolic acid, and cannabidiolic acid. Signalling with Gi/o, G12/13, Gq/11, Gs and beta-arrestin 1 and 2 pathways will be assessed using bioluminescence energy transfer (BRET) biosensors in human embryonic kidney 293 cells, as compared to CP55,940 and the endocannabinoids. We hypothesize that each phytocannabinoid will exhibit a unique signalling profile at each receptor. Preliminary results show different signalling between CB1R and CB2R for THCV, CBD, and CBC at Gi/o. Understanding phytocannabinoid pharmacology will help to elucidate the basis of the variety of effects produced by Cannabis sativa.

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Resolving Unchecked Inflammation in Atrial Fibrillation

Deanna Sosnowski, Roddy Hiram, Xiao Yan Qi, Kyla Bourque, Patrice Naud, Sandro Ninni, Darlaine Pétrin, Terence Hébert, Stanley Nattel

Objective: Persistent inflammation drives atrial fibrillation (AF), but inflammatory pathways in AF remain under-explored. NLRP3 inflammasome signalling is activated in atrial cardiomyocytes (CMs) in AF. Specialized Pro-resolving lipid Mediators (SPMs) reduce inflammation by binding to G protein-coupled receptors (GPCRs). LipoxinA4 (LxA4), an SPM, signals via the formyl peptide receptor-2 (FPR2). This study investigates CM-specific inflammatory signalling in AF and the role of LxA4-FPR2 signal transduction to attenuate chronic inflammation.

Hypothesis: LxA4 attenuates cardiac electrical and structural remodeling in AF by FPR2-mediated signalling, which targets the NLRP3 inflammasome in atrial CMs.

Methods: Adult dogs were atrial tachypaced at 600 beats per minute (AF) or remained in sinus rhythm for 3 weeks. Hearts from a separate cohort of healthy dogs were digested to isolate left atrial CMs that were paced in vitro at 1Hz or 3Hz for 24 hours in parallel. Assessment of NLRP3 inflammasome, cytokines, and FPR2 expression was done by SDS-PAGE and qPCR. hiPSCs were generated and differentiated into iPSC-CMs. iPSC-CMs were transfected with the ExRai-AKAR2-NLS biosensor to detect nuclear PKA activity, treated with LxA4, and imaged using an Opera Phenix microscope.

Results: FPR2 expression was reduced in AF canines hearts, while expression was unchanged in CMs paced at 3Hz. NLRP3 inflammasome levels and the cytokine, IL-1, were increased in paced CMs. Transcripts for FPR2 and other SPM-GPCRs were not expressed in iPSC-CMs. LxA4 treatment of iPSC-CMs did not influence nuclear PKA activity in the absence of FRP2.

Conclusions and Future Directions: CM-specific NLRP3 inflammasome signaling and FPR2 expression are disrupted in models of AF. Preliminary data suggests that LxA4 does not affect downstream PKA activity in iPSC-CMs in the absence of its receptor, FPR2. Attenuation of the NLRP3 inflammasome pathway via LxA4-FPR2 signalling mechanisms remains to be assessed.

Identification of the first neuroserpin inhibitory molecule for cholinergic maintenance in Alzheimer's Disease as a possible method of delaying clinical symptom onset

Benjamin Kannel, Sonia Do Carmo, and A. Claudio Cuello

In Alzheimer's Disease (AD), the atrophy and degeneration of the cholinergic neurotransmitter system, beginning with the basal forebrain cholinergic neurons (BFCNs), is central to the onset of symptoms. These BFCNs rely solely on mature nerve growth factor (mNGF) for their trophic support. The metabolic system controlling the availability of this mNGF is compromised in AD at preclinical stages, resulting in a lack of mNGF production and the inability to meet the trophic needs of these cells and thus they atrophy. Our lab hypothesizes that a pharmacological correction of this metabolism, through neuroserpin inhibition, can serve as a therapeutic route for normalizing the rate of endogenous mNGF production and thus trophic support to the BFCNs, delaying their atrophy and therefore delaying downstream cognitive decline. In this presentation I will go over the identification of target pockets within the protein neuroserpin, the in-silico identification of small molecules which can bind to these pockets, and the in-vitro testing of these small molecules for neuroserpin inhibitory capacity.

Steps in the development of assessing brain nicotinic receptor function in vivo during chronic nicotine exposure and withdrawal

Lucas Marques, Paul Clarke

It is widely thought that nicotine-dependent individuals smoke tobacco to obtain nicotine's stimulatory effect upon nicotinic acetylcholine receptors (nAChRs) in the brain, with withdrawal reflecting nAChR underactivity. However, there is considerable evidence that nicotine is a two-faced drug: while nicotine acts acutely as a nAChR agonist, during chronic exposure its predominant effect appears to be desensitization. In this view withdrawal would reflect overactivation by endogenous acetylcholine. While the functional status of nAChRs during withdrawal has predominantly been investigated in vitro and ex vivo, this project probes nAChR status in vivo in freely behaving rats using fibre photometry. Fibre photometry allows for the measurement of both neuronal calcium fluctuations (a readout of neuronal activity) and dopamine release dynamics.

This project has required a lot of troubleshooting, including failed attempts at using transgenic rats. The development of the project has led to the targeting of three brain areas- the ventral tegmental area (VTA) and medial geniculate nucleus (MGN) using the calcium indicator GCaMP and the nucleus accumbens shell (NAcSh) using the dopamine biosensor GRABDA. In the VTA, which predominantly expresses alpha4beta2 and alpha7 nAChRs, and MGN, which predominantly expresses the alpha7 subtype, the effects of chronic nicotine and subsequent withdrawal on calcium-related neuronal activity will be determined. In the NAcSh the effect of chronic nicotine and subsequent withdrawal on dopamine dynamics will be analyzed. It is hypothesized that chronic nicotine administration will reduce neuronal calcium and dopamine responses to acute in vivo nicotine challenge, indicating nAChR desensitization, and that during withdrawal the same neurons will produce an exaggerated response to an acute nicotine challenge, indicating nAChR supersensitivity.

The Effects of Bisphenol A and its Structural Analogs on the Expression of Gene Transcripts Involved in the Regulation of Steroidogenesis in KGN Human Granulosa Cells

Lama Iskandarani, Bernard Robaire, and Barbara F Hales

Exposure to bisphenol A (BPA), an endocrine disrupting chemical that is widely used in consumer products, is linked to alterations in ovarian steroidogenesis and changes in steroid hormone levels. Commercial use of BPA has decreased due to public pressure and government regulations, leading to its replacement with other bisphenols. However, little is known about the impact of these structural analogs of BPA and the mechanism(s) by which they may affect steroidogenesis. To determine the effects of some of these chemicals on progesterone production and on the key enzymes involved in the steroidogenic pathway, we cultured KGN human granulosa cells in the presence of vehicle, BPA, BPF, or BPS (5 or 20 μM) under both basal and dibutyryl-cAMP (Bu2cAMP) stimulated conditions. Under basal conditions, progesterone production was increased after exposure to BPA or BPF, whereas it was decreased after BPS exposure. The BPA-induced increase in progesterone was accompanied by an increase in STAR and CYP11A1 expression; there was no effect on these transcripts after basal BPF exposure. Exposure to BPA or BPS decreased progesterone production after Bu2cAMP stimulation, while BPF had no significant effect. All three bisphenols increased the expression of STAR and CYP11A1 after Bu2cAMP stimulation. In conclusion, BPA, BPF, and BPS all affected progesterone production in KGN cells. Further, these effects were accompanied by chemical-specific alterations in the expression levels of several gene transcripts. We suggest that BPF and BPS may not be responsible replacements for BPA in consumer products.

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Discovery of a Putative Urolithin A-Producing Operon in the Gut Bacteria

Reilly Pidgeon, Bastien Castagner

Urolithin A is a polyphenol derived from the multi-step metabolism of dietary ellagitannins by the human gut microbiota. In the host, urolithin A has been shown to reduce oxidative stress, promote mitophagy, and improve antitumor CD8+ T-cell immunity. Most adults harbor a microbiota capable of urolithin A production; however, the bacteria and enzymes that metabolize its precursor (urolithin C) are unknown. We discovered a putative urolithin C metabolism operon found in three *Enterocloster* species. Using liquid chromatography-mass spectrometry, we identified *E. asparagiformis*, *E. bolteae*, and *E. citroniae* as urolithin A producers in a panel of six *Enterocloster* species type strains. RNA sequencing of urolithin A producers revealed a three-gene molybdopterin oxidoreductase-containing cluster that was ~1000-fold upregulated in urolithin C-treated bacteria. Each gene of the three-gene cluster was expressed to a similar level by RNA-seq and RT-qPCR. Furthermore, RT-PCR analyses demonstrated that all three genes were transcribed on the same mRNA, indicating that these genes are part of an operon. Using comparative genomics, we found that this three-gene cluster was absent in the genomes of non-producer *Enterocloster* species. Growth experiments showed that urolithin C, but not urolithin A, delayed growth in *Enterocloster* species, indicating that metabolism may serve to detoxify catechol-containing compounds. Future experiments using heterologous expression in various hosts and CRISPR-based recombineering will be used to probe the physiological role of urolithin A production in gut bacteria.

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Characterization of myelin deficits in rats with Alzheimer's-like tauopathy

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Background: Alzheimer's disease (AD) is the most common form of dementia conceptualized as progressive pathological changes in grey matter: extracellular plaques composed of aggregated amyloid-beta (Aβ) and intracellular neurofibrillary tangles (NFTs) composed of phosphorylated tau. However, in addition to the grey matter in AD brains, there are significant abnormalities in white matter which are accompanied by glial pathological alterations. We postulate that the AD tauopathy is the main driver of the white matter pathology. However, there is no direct evidence on the relationship between tau pathology and demyelination. Therefore, our overarching goal is to evaluate whether and how a progressive tauopathy leads to myelin defects and relate to cognitive capabilities.

Method: To explore the effects of a progressive tauopathy on myelin health, we will apply our newly generated McGill-R955-hTau transgenic rats, which expresses the longest form of human tau with the P301S mutation. We will first characterize the cognitive function of transgenic rats at 12 months and 20 months in comparison to wild-type rats. Then we will examine region-specific alterations in myelination patterns and oligodendrocyte marker expression profiles as well as the main features of human tauopathies and neurodegeneration.

Results: Transgenic rats display age-dependent increases in human-specific tau, Phospho-Tau and conformation-Tau. The transgenic rats present cognitive impairments, brain atrophy, white matter loss and degraded myelin basic protein at 20 months of age. At 12 months of age, transgenic rats display cognitive impairments only in the social interaction test. Analysis of other myelin associated protein and oligodendrocyte expression profile is ongoing.

Conclusions: McGill-R955-hTau transgenic rats display tau pathology-driven defects in the white matter. More in-depth analyses are ongoing.

Cannabis Smoke Extract and Cannabis Dry Herb Vapour Induce Inflammation in Lung Epithelial Cells

Maddison Taylor Arlen, Emily Wilson, Rui Liu, Ryan Pusiak, D.H. Eidelman, Cory Harris, C.J. Baglole

The most common route of cannabis consumption is via inhalation of smoke. When cannabis is burned, cannabinoids, terpenes and a multitude of toxic combustion products are released. These include carcinogens, polycyclic aromatic hydrocarbons, and other pulmonary irritants that exacerbate inflammation. Dry herb vaporizers have been proposed as a safer alternative to smoking cannabis, as they heat the plant materials to release cannabinoids without producing harmful by-products of combustion. However, the safety profile of inhaled cannabis smoke and vapour remains poorly characterized, warranting further investigation in light of their increasing usage. We sought to develop cannabis smoke extract (CaSE) and cannabis vapour extract (CaVE) as in vitro surrogates of pulmonary exposure. The cannabinoids and combustion products were characterized by HPLC and GC/MS respectively. Higher levels of cannabinoids were released via smoking. Both burning and vaporizing cannabis emitted harmful combustion products. Treatment of the human lung epithelial cell line BEAS-2B with CaSE and CaVE for 6 and 24 hours significantly increased CXCL8, CYP1A1 and NQO1 mRNA levels, indicating a pro-inflammatory response to cannabis smoke and vapour containing the myriad of compounds inhaled by users. Cytokines will be analyzed via ELISA/multiplex assays as an indice for altered immune function. Despite the legalization of cannabis in Canada, the health consequences of inhaled cannabis products are poorly characterized. Further research is vital to ensure the safety of millions of Canadian consumers.

The Effects of E-cigarette Use on Cardiopulmonary Health

Vincenza Caruana, Koren K. Mann, Carolyn J. Baglole

E-cigarettes were designed to simulate the act of cigarette smoking without the injurious health effects of tobacco. Although e-cigarettes were intended to be a cessation tool for smokers, their sleek design and flavorings made them popular among teens and young adults. Yet, the health effects of e-cigarette use in non-smokers remains unclear. Several studies have correlated e-cigarette aerosols to increased inflammation and pathological conditions such as myocardial infarctions and organ fibrosis. Therefore, this project investigates the impact of a chronic, high-level e-cigarette exposure on multiple pulmonary and cardiovascular outcomes, including fibrosis and atherosclerosis. To model atherosclerosis, 5-week-old male and female C57BL/6J mice were injected with AAV-PCSK9 and fed a high fat diet. One week later, the mice were exposed to room air or the e-cigarette aerosol twice daily for 16 weeks. The commercially available e-cigarette brands STLTH and Vuse were used as they are among the most popular e-cigarette brands used today. After the exposure period, metal deposition, inflammatory and fibrotic markers in the lungs will be assessed. The extent of the atherosclerotic plaque and its constituents will be evaluated by en face oil red O staining, picrosirius red, α-smooth muscle actin, and MOMA-2 staining. These results have the potential to make significant impacts in understanding the long-term health consequences of vaping.

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Developing Methods for in vitro Toxicological Assessment of Inhaled Cannabis Products

Emily Wilson, Maddison Arlen, Rui Lui, Ryan Pusiak, Percival Graham, David Eidelman, Cory Harris, and Carolyn Baglole

Cannabis has been used for centuries to alleviate symptoms of chronic disease. The reported medicinal properties of cannabis are due to the presence of cannabinoids. Cannabis is commonly consumed via cannabis smoke which contains combustion products. There is an increasing interest in vaporizers, which heat the cannabis flower without burning. Here, we optimize methods for cannabis vapour extracts (CaVE) for submerged cell cultures and whole cannabis vapour exposures for air-liquid interface (ALI) cultures.

A549 cells were cultured in either submerged conditions or at ALI. ALI cultures were exposed to cannabis vapor using the SCIREQ® expoCubeTM. THC levels of the ALI basolateral compartment were assessed 24 hours after cannabis vapour exposure. This THC concentration was used to determine the volume of CaVE for submerged cell treatments. CaVE for submerged cells was created by bubbling cannabis vapor through ethanol. Cannabinoids were assessed using high-performance liquid chromatography. Media and mRNA were collected from submerged and ALI cell culture models. Cytotoxicity of cannabis vapour was assessed by lactate dehydrogenase assay. Induction of inflammatory cytokines was assessed via qPCR.

The expoCube delivers cannabis vapor to cells at ALI in a reproducible and controllable manner. Cannabis vapor shows potential to induce a proinflammatory response with increases in mRNA of CXCL8 and IL6. Submerged cell cultures are more susceptible to the inflammatory impacts of cannabis vapour with significantly increased induction of CXCL8 and IL6 compared to ALI cultures.

This is the first study to investigate the impact of vaporized cannabis products on the structural cells of the lungs. We also show the importance of cell culture model as cells at ALI are more sensitive to the cytotoxic effects of treatment compared to submerged cultures. Overall, this work will set the foundation for future in vitro work with inhaled cannabis products.

Acknowledgements | Mitacs Accelerate

Sex as a modulator of arsenic-induced immune cell heterogeneity within atherosclerotic plaque in apolipoprotein E -/- mice

Ayse Nazli Zengin, Kiran Makhani, Nivetha K. Subramaniam, Braeden Giles, Natascha Gagnon, and Koren K. Mann

Arsenic (As) is an internationally recognized environmental contaminant affecting many countries including Canada. Although municipal water is regulated, many Canadians live in rural areas and are dependent on groundwater where As levels are not regulated. As exposure is linked to a myriad of adverse health effects, such as cancer and cardiovascular diseases (CVDs), especially atherosclerosis. Atherosclerosis is a CVD characterized by narrowing of vessels due to fibrous-fatty build up referred to as plaques. Even after adjusting for smoking, diet and hypertension, As remains a risk factor for atherosclerosis but the mechanisms by which As induces atherosclerosis is still unknown. With single cell RNA sequencing (scRNAseq), we previously showed that 200 ppb As in drinking water changes the cellular composition of atherosclerotic plaques in male apoE-/- mice that were fed a high fat diet (HFD). Moreover, epidemiological data shows CVDs present themselves differently between men and women. Thus, we hypothesized that sex could act as a modulator of As-induced immune cell heterogeneity within atherosclerotic plaque. To test this hypothesis, 5-week-old female apolipoprotein E knock-out (apoE-/-) mice were exposed to either 200 ppb As or to tap water while given a HFD. At the end of 13 weeks, aorta and hearts were harvested. The size of the plaques was assessed by oil red-O staining in the aortic arch. Our preliminary data shows that, in female mice, As increases lesion size in the aortic arch in contrast with what is previously seen in males, where there is no significant difference between control and As groups. This finding suggests that arsenic treatment is affecting plaque properties differently depending on sex. Further assessment of different plaque constituents and their contribution to the As-enhanced atherosclerotic plaque by scRNAseq and the PhenoCycler multiplex imaging technique may also help explain As-induced immune cell heterogeneity in both sexes.

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Investigating a Novel Transfection Method for the In Vitro Screening of saRNA Drugs

Monika Kojic, Maureen McKeague

Acute myeloid leukemia (AML) is an aggressive and highly heterogenous blood cancer characterized by the excessive production of abnormal blood cells. Despite several available treatment options, the five-year survival rate for AML is still only 29%. The most common therapeutic strategy used to treat AML is chemotherapy, sometimes used in conjunction with targeted therapies, but not without debilitating adverse effects. Small activating RNAs (saRNAs) have garnered interest as potential anti-cancer therapeutics and are currently being investigated as an alternative treatment for the upregulation of critical genes involved in rescuing AML pathogenesis. However, the efficiency of current transfection methods used for the development and in vitro screening of these saRNA drugs is hindered by high cell death, high cost, and difficulty enveloping saRNAs. This study will examine the suitability of a novel transfection method, ViaFect, for the in vitro screening of saRNA drugs.

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The Th17 inflammation axis as a potential mediator of persistent fatigue symptom in post-COVID-19 condition

Jérôme Bédard-Matteau, Simon Rousseau

COVID-19 has been a concerning topic, with almost 500 million cases worldwide in the past few years. As a result, the scientific community has become more aware of the multiple long-term effects of this viral infection. Indeed, many neurological symptoms persist many months following the initial infection, including chronic fatigue syndrome (CFS). In collaboration with the Biobanque Québécoise de la COVID-19 (BQC19), this research project tries to explore the effect of IL-17F, a cytokine involved in the immune response to SARS-CoV-2, the virus responsible for COVID-19 and its relationship with persistent fatigue reported by patients. Our findings suggest that exposure to IL-17F causes actin remodelling in endothelial cells. Whether this actin reorganization is physiological or pathological remains to be determined but could be a contributor to endothelial dysfunction. We propose a potential way to modulate this remodelling of the actin by pre-treating the cells with two MAPK inhibitors: PD184352 and BIRB 796, before exposing them to IL-17F. In order to assess changes in endothelial functions, we are using functional assays such as neutrophils binding assays, permeabilization as well as looking and measuring the expression of specific cell surface markers such as ICAM-1 and P-selectin. This work is essential as we start to gain much knowledge on the long-term effects of COVID-19; we are truly starting to see the many effects of the inflammatory response involving many cytokines such as IL-17.

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Characterisation of an Additional As3MT Function in Mouse Embryonic Fibroblasts

Andrew Little, Cynthia Guilbert, Koren Mann

Arsenic poses a public health risk given the sequelæ of chronic exposure: cardiovascular disease, cancer, and diabetes. Arsenic is metabolised through methylation by arsenic (3) methyltransferase (As3MT). Arsenic methylation is the only known function of As3MT, and reciprocally, arsenic is methylated only by As3MT. Thus, As3MT knockout mice exhibit impaired arsenic methylation and slower clearance. Preliminary data supports the hypothesis that As3MT has additional functionality: 1) the basal phenotype of As3MT–/– cells is a stress response and slower growth rate; and 2) GWAS showed that the As3MT locus is associated with ischæmic heart disease and schizophrenia. To investigate the hypothesis about an additional As3MT function, proteomic and functional differences associated with As3MT expression were characterised. Protein expression was quantified in mouse embryonic fibroblasts (MEFs) by liquid chromatography-mass spectrometry (LC-MS) to evaluate As3MT-dependent differences in parallel with changes induced by 24-hour 1 μM arsenite exposure. Pathway enrichment analysis suggested that basal differences between wild type and As3MT–/– MEFs correspond with cell adhesion, cytoskeleton, endocytic trafficking. These data were complemented by immunoprecipitation-mass spectrometry of wild type and As3MT–/– AML12 hepatocytes, which also pointed to cell adhesion and trafficking. The LC-MS hits were validated by immunoblots that confirmed depletion of vimentin and β-catenin and upregulation of HO-1, Vps35, and myoferlin in As3MT–/– MEFs. Reconstitution with As3MT, but not a methylation-dead copy of As3MT, completely restored vimentin expression and growth rate. These data indicate that vimentin expression is basally regulated by As3MT, and that this additional function depends on its methylation activity. Wound healing, collagen contraction, and immunofluorescence experiments will explore whether the As3MT-dependence of vimentin expression translates to functional differences.

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SPI1-targeted RNA duplex increases PU.1 expression in Acute Myeloid Leukemic cells

Olivia Kovecses, Iris Harmsen, Maureen McKeague

Acute myeloid leukemia (AML) is an aggressive blood cancer with a mortality rate of over 70%. AML is characterized by arrested differentiation and continued division of immature myeloid cells. Normal hematopoietic differentiation is regulated by the relative expression of key transcription factors. Most notably, PU.1 (encoded by the SPI1 gene) is a master myeloid regulator which is transcriptionally silenced in >50% of AML patients. In fact, rescue of PU.1 expression in AML models removed the differentiation block and promoted apoptosis of leukemic cells. Therefore, targeting transcription factor PU.1 is a promising AML treatment strategy. Nonetheless, there are no drugs currently available to target and upregulate gene expression. Small activating RNAs (saRNA) are a novel discovery in the field of RNA therapeutics that are capable of reversibly increasing gene expression by targeting the promoter region of the gene of interest. saRNA-promoter interactions lead to the recruitment of transcription initiation factors and histone modifications that induce long-term gene upregulation. After designing and screening multiple SPI1-targeted saRNAs, we have identified a candidate saRNA capable of increasing SPI1 mRNA and protein expression in two AML cell lines, MV4-11 and OCI-AML3. Future work will focus on assessing the therapeutic effects of this upregulation on differentiation and proliferation in in vitro and ex vivo models of AML.

HIV-1 Maturation Inhibitors & Packaged Inositol Phosphates

Justin Meneses, Robert Dick, Bastien Castagner

The most recent UNAIDS global update has estimated that there are currently 38.4 million people living with human immunodeficiency virus (HIV) with an expected 1.5 million new infections each year. Of these 38 million patients, there are approximately 26 million accessing the standard of care treatment: highly active antiretroviral therapy. While the combination of antiretroviral therapeutics has largely been effective at treating HIV, there is a continuing need for new long-acting drugs that target novel mechanisms in viral replication to overcome problems with patient tolerability, dose adherence, and emerging antiretroviral drug resistance. To this end, HIV maturation inhibitors (MIs) are a newly developed class of antiretroviral therapeutics that are distinguished by their unique target and mechanism of action. MIs work by stabilizing the capsid-spacer peptide 1 region of the immature polyprotein Gag and preventing viral protease mediated cleavage at this site, which is required for maturation and therefore infectivity. Recently, inositol hexakisphosphate (IP6) has been characterized as a structural co-factor in HIV that is critical to viral maturation and replication. The IP6 binding pore is located directly above the first-in-class MI (bevirimat) binding site. However, it remains unclear if the IP6 binding pocket can be exploited by novel HIV MIs. We hypothesize that extending MIs into the IP6 binding site will simultaneously inhibit Gag cleavage and IP6 packaging. This strategy will provide dual inhibitory mechanisms to lead to more potent MIs that are less susceptible to the development of resistance. The synthesis of IP analogues is also of interest as biochemical probes as they are critical cofactors in several different proteins. We have synthesized extended MIs and expanded the methodology of IP analog synthesis and in collaboration with Dr. Robert Dick at Cornell University, are exploring their utility as therapeutics and as probes.

Maturing and phenotyping patient-derived iPSC-cardiomyocytes to understand dilated cardiomyopathy

Alyson Jiang, Kyla Bourque, Cara Hawey, Karima Alim, Ida Derish, and Terence E. Hébert

The second most common cause of heart failure is dilated cardiomyopathy (DCM), a disease affecting the heart's ability to deliver oxygenated blood to the body. Diagnosing and treating DCM remains difficult due to its diverse causes and clinical presentations. We hypothesize that characterizing phenotypic properties and underlying molecular mechanisms of DCM patient-derived induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) compared to controls will help stratify DCM patients into defined disease subtypes. We aim to 1) induce more mature phenotypes in our DCM iPSC-CMs, 2) characterize differences in cellular morphology between DCM and healthy iPSC-CMs and, 3) investigate differences in G protein-coupled receptor (GPCR) signaling profiles between DCM and healthy iPSC-CMs. Two metabolic maturation media were tested: 1) a low glucose medium with palmitate, T3 hormone, a PPARα agonist, and dexamethasone and 2) a low glucose medium with albumin-bound fatty acid, creatine, L-carnitine, and taurine. Morphological profiles will be characterized using high-content imaging and multiplexing dyes staining various organelles. Differences in GPCR signaling profiles were characterized using biosensor-based approaches. The first maturation medium resulted in iPSC-CM death whereas the second medium resulted in increased mature gene expression. We optimized a protocol to characterize nuclear, membrane, and mitochondrial morphology in live cells and sarcomere organization in fixed cells. Cytosolic PKA activity recorded in a DCM patient line revealed distinct signaling profiles at the single-cell level. Mature iPSC-CMs will provide us with a more disease-relevant model to generate DCM patient clusters. Generated morphological phenotypes will be used in combination with calcium handling and GPCR signaling phenotypes to cluster DCM patients. Overall, we anticipate this workflow to pave the way for more personalized treatments and to improve DCM patient outcomes.

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Organophosphate esters disrupt steroidogenesis in KGN human ovarian granulosa cells

Xiaotong Wang, Elaine Lee, Barbara F Hales, Bernard Robaire

Organophosphate esters (OPEs), used extensively as flame retardants and plasticizers, are ubiquitous in the environment. Data from previous studies suggest that OPEs are endocrine active and thus may have detrimental effects on female fertility. Here, we tested the hypothesis that OPEs alter the steroidogenic ability of ovarian granulosa cells by dysregulating the expression of transcripts in steroid and cholesterol synthesis pathways. Immortalized human KGN granulosa cells were exposed (1–50 μM) for 48h to one of six OPEs [triphenyl phosphate (TPHP); tris(methylphenyl) phosphate (TMPP); isopropylated triphenyl phosphate (IPPP); tert-butylphenyl diphenyl phosphate (BPDP); tributoxyethyl phosphate (TBOEP)] or a predominant PBDE [2,2',4,4' tetrabromodiphenyl ether (BDE-47)] in the presence or absence of a steroidogenic stimulus, Bu2cAMP. While exposure to BDE-47 had no effects, OPEs significantly increased the basal production of progesterone (P4) and estradiol-17ß (E2) and inhibited the Bu2cAMP-stimulated P4 and E2 synthesis. qRT-PCR analyses revealed that OPEs (≥ 5 μM) increased the basal expression of critical genes involved in steroidogenesis (STAR, CYP11A1, CYP19A1, and HSD3B2); upon stimulation, the expression of all genes tested was generally downregulated. An overall inhibition in cholesterol biosynthesis was induced by OPEs, characterized by a downregulation in HMGCR and SREBF2 expression. In summary, OPEs exhibited higher potency than BDE-47 to perturb steroidogenesis in KGN granulosa cells by targeting the expression of key steroidogenic enzymes and cholesterol transporters. OPEs also disrupt cholesterol homeostasis in KGN cells; this may contribute to alterations in steroid production. Thus, OPE exposures may adversely affect female reproduction by altering the function of granulosa cells.

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mGluR5 dysfunction underlies mGluR-LTD deficit in the absence of synaptopodin

Pei You Wu, Yanis Inglebert, R. Anne McKinney

Synaptopodin (SP) is an actin-associated protein found only in a subset of excitatory synapses, mainly in the larger and more stable dendritic spines of the telencephalic neurons. It is necessary for the formation of spine apparatus, an organelle located at the base of dendritic spines that is involved in local protein synthesis and calcium regulation in individual spines. It has been shown that SP knock-out mice (SPKO) exhibits normal synaptic transmission and dendritic spine density. However, synaptic plasticity such as NMDAR-LTP was found impaired in SPKO. It is currently unknown whether SP is involved in other types of plasticity such as metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD).

mGluR-LTD is critically involved in learning and memory formation in brain. Enhanced mGluR-LTD is associated with Fragile X syndrome, where an elevated level of SP is also observed. To understand the role of SP in mGluR-LTD, we used electrophysiology technique and found that hippocampal mGluR-LTD is impaired in SPKO. Furthermore, we found that mGluR-LTD becomes mGluR5- and protein synthesis-independent in SPKO. Lastly, we show evidence that, although mGluR-LTD is induced primarily through postsynaptic events, the lack of SP switched mGluR-LTD to be presynaptically driven.

High content imaging analyses of the effects of six organophosphate esters in the HepG2 cell line

Dongwei Yu, Barbara F. Hales, Bernard Robaire

Organophosphate esters (OPEs) are used as flame retardants and plasticizers. OPEs are easily released into the environment. Six of the major OPEs detected in Canadian house dust are: tris(2-butoxyethyl) phosphate (TBOEP), tris(1-chloro-2-propyl) phosphate (TCIPP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), triphenyl phosphate (TPHP), tris(methylphenyl) phosphate (TMPP), and isopropylated triphenyl phosphate (IPPP). We used a high content imaging approach to test the hypothesis that these OPEs affect liver HepG2 cells. This was followed by toxicological prioritization index (ToxPi) analyses to compare their potencies. HepG2 cells were exposed for 48h to vehicle control (DMSO) or each OPE (0.001-100μM). Cells were screened using fluorescent dyes to elucidate effects on cytotoxicity, oxidative stress, mitochondria, lipid droplets and lysosomes. The relative cytotoxicities of the OPEs were: IPPP>TMPP>TPHP>TDCIPP>TCIPP=TBOEP; thus, triaryl OPEs were more cytotoxic to HepG2 cells than non-triaryl OPEs. TBOEP, TCIPP, TMPP and TPHP reduced the labelling for reactive oxygen species. Exposure to TCIPP, TDCIPP, TMPP, IPPP or TPHP significantly increased total mitochondrial intensity. Exposure to IPPP increased the total area of lipid droplets. TBOEP, TCIPP, TMPP, IPPP and TPHP significantly increased the labelling of lysosomes. Using ToxPi analyses, the integrated relative potency ranking for OPEs was: IPPP>TPHP>TBOEP>TMPP>TDCIPP>TCIPP. Since the organelles that were most affected, lipid droplets and lysosomes, are involved in the autophagy pathway, we used immunofluorescence staining with PLIN2, LAMP1 and p62 to investigate the impact of OPEs on autophagosomes. Preliminary results show that IPPP, in combination with an autophagy inhibitor, increased the accumulation of p62, suggesting that it may interfere with autophagosome formation and/or degradation. Together these data suggest that the OPEs have differential effects on a variety of endpoints in HepG2 cells.

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Effects of organophosphate esters (OPEs) used as flame retardants and plasticizers on the function of H295R human adrenal cells

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OPEs that are used extensively as flame retardants and plasticizers and are ubiquitous in the environment. Our previous assessment of the phenotypic characteristics of H295R adrenal cells revealed that exposure to OPEs increased the area of lipid droplets. However, whether their steroid-producing function was affected remains unclear. The goal of this study was to test the hypothesis that OPEs affect steroid production in H295R cells. H295R cells were exposed for 48h to one of three commonly used OPEs: isopropylated triphenyl phosphate (IPPP); triphenyl phosphate (TPHP); or tris(methylphenyl) phosphate (TMPP) at 1 μM or 5 μM. We assessed the steroidogenic ability of H295R cells in the presence or absence of a steroidogenic stimulus, forskolin. Basal cortisol production was increased after exposure to IPPP but decreased after TPHP or TMPP exposure; the response of cells to forskolin was not affected by OPEs. Further, RT-qPCR results showed that the IPPP-induced increase in cortisol level was accompanied by an upregulation in STAR expression; in contrast, TPHP and TMPP induced a downregulation of STAR expression. The expression of CYP11B1, the key enzyme involved in cortisol biosynthesis, was strongly upregulated by IPPP under basal and stimulated conditions; TPHP affected CYP11B1 expression under basal conditions only and TMPP had no effect. All three OPEs altered the expression of rate-limiting enzymes involved in cholesterol biosynthesis (HMGCR) and steroidogenesis (CYP11A1) as well as an upstream regulator (NR5A1). Thus, OPE exposures may adversely affect the function of adrenal cells through disruption of the cholesterol and steroid biosynthesis pathways.

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Impaired microglial heme metabolism prevents retinal vascular remodeling and impairs vision in proliferative retinopathy

Tapan Agnihotri, Nicholas Kim, Gael Cagnone, Jin Sung Kim, Emilie Heckel, Sheetal Pundir, Perrine Gaub, Florian Wunnemann, Walter Szarek, Hyman Schipper, Jean-Sebastien Joyal

Retinopathy of prematurity (ROP) is a leading eye disease of premature newborns, characterized by vaso-obliteration (VO), retinal ischemia, and pathological neovascularization. Retinal microglia are resident phagocytes that scavenge injured tissues and remodel the ischemic neurovascular retina in ROP. In a classical murine model of ROP, the oxygen-induced retinopathy (OIR) model, ferroptosis and iron metabolism were amongst the most upregulated pathways in pathological neovessels and retinal microglia by single-cell RNA sequencing. The primary source of iron is heme contained in hemoglobin and the mitochondrial respiratory chain, which is critical to oxygen delivery and consumption. Here we show that disrupting heme metabolism in activated microglia prevents retinal remodeling and impairs vision. Heme is degraded by heme-oxygenase 1 (Hmox1), which was almost exclusively expressed in retinal microglia exposed to OIR. We, therefore, depleted Hmox1 in microglia (LysM-Cre conditional mice) and exposed our transgenic mice to the OIR model. Microglial Hmox1 depletion slowed retinal revascularization of the superficial (P17) and deep (P19-21) vascular plexi and slowed neovascular tuft regression (P19). Mutant mice showed increased microglial counts, largely surrounding ischemic and neovascular retinal regions, and morphological microglial changes associated with an activated state. Delayed revascularization and microglial activation correlated with impaired vision by electroretinogram (ERG); reduced a-wave amplitudes were observed in scotopic ERG of conditional Hmox1-deficient retinas. Hence, heme recycling and iron metabolism by microglial phagocytes require HMOX1 for retinal revascularization. Inefficient heme recycling critically impairs retinal regeneration and vision, emphasizing its importance to retinal health.

Characterizing the role of KCTD proteins and Gβγ subunits in regulating the assembly and function of the GPCR landscape

Robert Rizk, Darlaine Pétrin, Dominic Devost, Terence E. Hebert

G protein-coupled receptors (GPCRs) play a crucial role in regulating cardiac physiology and have been extensively studied for their pharmacological potential in treating various cardiovascular diseases (CVD), such as hypertension and heart failure. Upon ligand binding, GPCRs undergo conformational changes, leading to their specific coupling with effector partners, including G proteins (Gα, Gβ, and Gγ subunits) and β-arrestin, to initiate downstream signaling. Gβ and Gγ subunits can form diverse dimeric pairs, resulting in a pool of Gβγ complexes that have now emerged as key mediators of receptor signaling in their own right. The different Gβγ dimer combinations mediate unique intracellular signaling pathways, thereby regulating the GPCR landscape. Despite the crucial role of the Gβγ pool in regulating GPCR signaling, the mechanisms that regulate this pool remain unclear. However, recent studies have shed light on the role of potassium channel tetramerization domain (KCTD) proteins in this regard. Our previous work utilizing tandem affinity purification has demonstrated that Gβγ interacts with a subset of KCTD proteins. Moreover, it has been shown that KCTD proteins act as adaptors for a multi-subunit CUL3-RING ubiquitin ligase (CRL3), which suggests that they play a role in regulating the number and type of Gβγ subunits in a given cell through proteasome-dependent degradation. Here, we show that KCTD proteins regulate the level of Gβ proteins through the CRL3-ubiquitin ligase complex, which can lead to changes in both the GPCR transcriptional profile and downstream signaling. Our TAP pulldown experiments showed that ΔKCTD5 cells have reduced Gβγ associated ubiquitination which correlated with an increase in the level of Gβ proteins compared to the wildtype cells. Moreover, our RNA-seq analysis of different KCTD protein knock-out cell lines revealed altered expression of various protein actors across the GPCR signaling landscape. These findings, taken together, bring us closer to understanding a key regulatory component for Gβγ-dependent signaling and its contribution to the overall GPCR signalosome as well as a potential role for this mechanism in cardiovascular homeostasis.

Identification and Characterization of Two Novel Enhancers for Human FSHB

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Follicle-stimulating hormone (FSH) from pituitary gonadotrope cells regulates ovarian and testicular function. FSH is a dimeric glycoprotein, and synthesis of its β subunit (FSHB) is ratelimiting in production of the mature hormone. FSHB expression is stimulated by activin class ligands (hereafter, activin). Activin regulates murine Fshb transcription via SMAD3/4 and forkhead box L2 (FOXL2), which bind to the proximal promoter. Mechanisms of activin regulated human FSHB transcription are less well-described. We identified at least three putative enhancers that can increase basal and activin-stimulated murine Fshb promoter activity in these cells. Using single nucleus ATAC-seq analysis of human pituitaries, we identified two regions of open chromatin upstream of the FSHB gene in human gonadotropes that correspond to two of the four putative enhancers in mouse (hereafter referred to as ENH2 and ENH3). ENH2 or ENH3 conferred activin responsiveness to the human FSHB promoter-reporter in LβT2 cells. ENH3 had higher enhancer activity than ENH2. Two single-nucleotide polymorphisms (SNPs), rs11031005 and rs11031006, occur in ENH2. Women with the minor allele of either or both SNPs (rs11031005 T>C minor allele frequency (MAF) = 0.112 Global, rs11031006 G>A, MAF = 0.132 Global) have decreased FSH levels. We are currently examining the effects, if any, of the SNPs in ENH2, in combination and separately, in reporter assays. Collectively, the data indicate that there are two highly conserved enhancers that can regulate FSHB/Fshb transcription in mice and humans. SNPs in these enhancers may contribute to variation in FSH levels in humans.

Pulmonary Toxicity of E-cigarettes in a Murine Model of Allergic Asthma

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Electronic (e-) cigarettes are battery-powered devices that produce an aerosol by heating up a liquid composed of nicotine, flavouring agents, propylene glycol and vegetable glycerin (PGVG). In recent years, e-cigarettes have gained popularity among adolescents, raising concerns over the potential adverse effects in youth with respiratory diseases such as allergic asthma. In this study, we aimed to determine how a sub-chronic exposure to a flavoured aerosol affected airway and pulmonary inflammation in a murine model of allergic asthma. Male and female C57BL/6 mice were exposed to a mint flavoured aerosol containing nicotine for 14 days. The control groups were exposed to air only or PGVG only. Then, three mice from each exposure group were euthanized immediately after the last aerosol exposure to characterize immediate effects of inhaled aerosols. The remaining mice from each group were sensitized and challenged with ovalbumin (OVA) to induce inflammation, a key feature of an asthmatic phenotype (Days 0, 7, 14-16), or treated with PBS as the control. We collected serum, the bronchoalveolar lavage (BALF) and the lung tissue (Days 17, 19) to measure total serum IgE and characterize inflammation via flow cytometry. Immunophenotyping of BALF immune cells revealed infiltration of eosinophils in the BALF of OVA-treated mice; in contrast, there were no eosinophils in the BALF of PBS-treated mice. Similarly, among air-exposed mice, the frequency of eosinophils in the lung tissue was significantly higher in OVA-treated mice compared to air-exposed mice treated with PBS. However, there was no difference in eosinophils frequency among PGVG- and JUUL-exposed mice treated with OVA or PBS. Serum IgE was higher in OVA-treated mice compared to the respective PBS control group, but there was no difference between air- and JUUL-exposed mice. This study is the first to investigate how a prior exposure to e-cigarette aerosols impacts the pulmonary inflammatory response in a murine model of allergic asthma. These results demonstrate that a past exposure to e-cigarettes did not significantly alter the immune cell composition of the BALF and lung tissue, and did not significantly exacerbate the humoral response in an ovalbumin-induced model of allergic asthma. Future studies should thus focus on the effects of a concurrent e-cigarette aerosol exposure in murine models of asthma.

Predictive Biomarkers for Efficacy of Cytarabine in Acute Myeloid Leukemia Therapeutics

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Acute Myeloid Leukemia (AML) is the most common form of Leukemia in adults. The current front-line induction therapy includes cytarabine (ara-C); however, the 5-year overall survival rate is only ~20%. Our objective is to understand why ara-C works in some patients and to identify biomarkers of treatment response. We have found that the mechanism of programmed cell death is a clinically relevant marker of cytarabine treatment efficacy. Activation of apoptosis by ara-C has been well characterized, but our work reveals that AML cells treated with ara-C undergo caspase-independent programmed cell death. Specifically, by assessing nuclear fragmentation, caspase activation, PARP-1 inhibition, and differentiation markers, we found that parthanatos, a PARP-1 dependent and caspase-independent programmed cell death pathway, is activated by ara-C in AML. Importantly, our double-blinded evaluation of AML patient samples ex vivo revealed that the presence of parthanatos features is clinically relevant and correlates with 3-fold longer overall patient survival. Indeed, there are several studies investigating PARP inhibitors in AML treatment. However, an important outcome of this work is that PARP inhibition, combined with ara-C treatment, may lead to unfavourable treatment responses in certain patients (FAB M4 and M5) by inactivating the parthanatos pathway.

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Understanding the Effect of Transforming Growth Factor-beta (TGF-β) on the Phenotypic and Functional Changes of Pulmonary Neutrophils

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Individuals suffering from cutaneous burn injuries are more susceptible to pulmonary infections, especially from Pseudomonas aeruginosa (Pa). Given that Pa is multidrug-resistant, alternative treatments are in need. Mice become susceptible to Pa 3-24 hours after burn injury. Within this period, the Transforming Growth Factor Beta (TGF-β) was upregulated. A Program Death Ligand-1 (PD-L1) positive pulmonary neutrophil population was observed to cluster in the lung vasculature. By neutralizing TGF-β or blocking PD-L1, burn-injured mice were less susceptible to Pa infection, and their lung neutrophils no longer clustered. Our preliminary studies suggest a potential TGF-β - PD-L1 axis on lung neutrophils, which is crucial for infection susceptibility.

Based on our preliminary studies, we hypothesize that TGF-β alone induces PD-L1 expression on pulmonary neutrophils, leading to neutrophil clustering in lung vasculature, which limits their ability to migrate to the site of infection and clear bacteria.

Human Leukocyte-60 (HL-60) derived human neutrophils were treated with 500 pg/mL of TGF-β and assessed with fluorescent immunohistochemistry and gentamicin protection assay. Adult CF-1 mice were intravenously given 500 pg TGF-β. 17 hours later, their lungs are harvested for whole-mount microscopy. A transgenic mouse model with neutrophil-specific Tgfbr2KO was generated. We will apply the thermal injury model, flow cytometry, and intravital microscopy.

TGF-β drives PD-L1 expression and clustering activity on HL60-derived human neutrophils in-vitro. TGF-β treated HL60-derived neutrophils phagocytose more Pa but cannot clear Pa intracellularly. Following a TGF-β treatment, mouse pulmonary neutrophils upregulate PD-L1 expression and form clusters. Clustering predominantly occurs in neutrophils but not in other immune cells. Similar lung vessel diameters observed between TGF-β and vehicle treatment suggested that clustering is not a result of restrained vessels.

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Alarmin responses in stressed human microglia

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Damage-associated molecular patterns (DAMPs) are molecules that serve as alarmins, alerting the organism of damage or infection. High-mobility group box 1 (HMGB1) is a DAMP that has been shown to play a key role in the progression of neuroinflammation and neuronal cell death in stroke. Both the redox state and post-translational modifications of HMGB1 influence its subcellular localisation, extracellular release, and function. Acetylated HMGB1 (acHMGB1) is preferentially released into the cytosol and extracellular space while being prevented from nuclear re-entry, activating pro-inflammatory cytokines. It has been observed that overexpression of the 72 kDa heat-shock protein (Hsp72), also a DAMP, suppresses the release of HMGB1, however, it was not determined how and if these two proteins interacted, or if this effect was present for acHMGB1. The objective of our study is to determine the abundance, subcellular localisation and extent of interaction between HMGB1 and Hsp72 under hypoxic stress and metabolic impairments in human microglia To mimic hypoxia in ischemic stroke, we used an oxygen-glucose deprivation (OGD) model. We found that under OGD conditions, both non-acetylated HMGB1 and acHMGB1 abundance was significantly reduced in the nucleus of human microglia and macrophages, while Hsp72 cytosolic abundance in microglia slightly increased under OGD treatment. Extracellular DAMPs have been investigated but interactions between HMGB1 and Hsp72 have not been reported. A proximity ligation assay was used to determine if HMGB1 and/or acHMGB1 interacted with Hsp72. Our data suggest that both forms of HMGB1 interact with Hsp72, but their interactions are differentially affected by stressed conditions. Interestingly, a natural compound, resveratrol, enhances the abundance of Hsp72 and modulates the interaction between HMGB1/AcHMGB1 and Hsp72. Molecular modelling is currently ongoing to define the sites and types of binding of resveratrol to HMGB1 and Hsp72.

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AhR Signaling in Macrophages Protects Against Cigarette Smoke-Induced Lung Inflammation

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BACKGROUND. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that responds to chemicals in cigarette smoke (CS), such as benzo[a]pyrene. Upon agonist binding, AhR is transported to the nucleus and induces transcription of target genes such as cytochrome p450 CYP1A1; then, AhR is exported from the nucleus and undergoes proteasomal degradation. Although AhR has been shown to protect the lungs against CS-induced lung inflammation, its mechanism is unclear. We hypothesize that AhR protects against CS through its signaling in macrophages.

METHODS. THP-1 monocytes were differentiated into macrophages using phorbol 12-myristate 13-acetate (PMA) and treated with cigarette smoke extract (CSE) or the endogenous AhR agonist 6-formylindolo[3,2-b]carbazole (FICZ). Expression of AhR was assessed via western blot, and gene expression of CYP1A1 was assessed via RT-qPCR. In addition, single cell RNA-sequencing (scRNA-seq) data from the lungs of non-smokers and smokers were assessed for AhR-dependent gene expression.

RESULTS. THP-1 monocyte differentiation into macrophages increased AhR expression, and treatment with CSE or FICZ decreased AhR expression. However, CYP1A1 mRNA was undetected in THP-1 monocytes and macrophages, both at baseline and after treatment with FICZ or CSE. Consistent with these results, scRNA-seq data demonstrated AHR mRNA expression across human lung macrophage populations, but CYP1A1 mRNA expression was negligible.

DISCUSSION. In macrophages, AhR was downregulated after treatment with CSE or FICZ, consistent with the canonical AhR signaling pathway in which AhR nuclear translocation is followed by degradation. However, AhR activation from treatment with CSE or FICZ did not induce CYP1A1 mRNA, raising the possibility that AhR has unique DNA binding behaviors in macrophages compared to previously studied cell types.

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Mast cell activation contributes to pathological angiogenesis in a model of choroidal neovascularization

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Background: Choroidal neovascularization (CNV) is a serious complication of various eye diseases, including wet age-related macular degeneration (AMD). The loss of choroidal vascular homeostasis in favor of a proangiogenic microenvironment promotes proliferation and leakage of neovessels. This leads to irreversible damage and death of photoreceptors, resulting in blindness. Inflammation and degeneration of the retinal pigment epithelium (RPE) are considered to be the main contributors to AMD pathogenesis. Recent studies have reported the accumulation and degranulation of mast cells in the compromised choroid of patients with wet AMD. When activated, mast cells secrete a variety of biologically active mediators, including inflammatory cytokines and proteolytic enzymes such as tryptase. However, the precise role of mast cells in the pathogenesis of AMD remains largely unknown. The purpose of this study is to determine the role of mast cells in pathological CNV.

Methods: Choroidal CNV was induced using laser photocoagulation. Mast cell infiltration was assessed by avidin and tryptase staining. CNV area was determined using lectin staining on RPE/choroid flat mounts. Denatured collagen was determined using collagen hybridizing peptide (CHP). The angiogenic effect of mast cells was examined using mast cell-deficient Kitwsh/wsh mice and a mast cell stabilizer, ketotifen fumarate. Mice received intraperitoneal injections of ketotifen fumarate, tryptase inhibitor nafamostate mesylate, or vehicle daily and were sacrificed at day 7 post-laser burn.

Results: Avidin- and tryptase-positive mast cells were found at the injury site three days post-laser burn. RNA-seq data on RPE/choroid complex at this timepoint revealed an enrichment in genes involved in extracellular matrix organization remodeling, suggesting a potential involvement of choroidal mast cells in this process. CNV lesion and CHP binding were attenuated in mast cell-deficient Kitwsh/wsh mice. Likewise, ketotifen fumarate and tryptase inhibition, using nafamostate fumarate were effective in decreasing the CNV area. Tryptase and conditioned media from activated peritoneal mast cells exerted proangiogenic effects ex vivo on choroidal vascular sprouting, and in vitro on choroidal endothelial migration and tube formation.

Conclusion: This study shows that mast cells confer proangiogenic properties in the choroid and are a potential target for the treatment of CNV.

Androgens upregulate pathogen-induced placental innate immune response

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Group B Streptococcus (GBS) is a leading cause of placental infection, termed chorioamnionitis. Chorioamnionitis is associated with an increased risk of fetal morbidities including life-long neurobehavioral impairments, such as autism spectrum disorders, cerebral palsy or attention deficit hyperactivity disorder, which are more prominent in males than females. In a pre-clinical model of chorioamnionitis, a greater inflammatory response was observed in placenta associated with male rather than female fetuses, correlating with the severity of subsequent neurobehavioral impairments. The reason for this sex difference is not understood. Our hypothesis is that androgens upregulate the placental innate immune response in male fetuses and play a role in GBS-induced autistic traits of male offspring. Therefore, we tested the effect of androgen blockade in alleviating the molecular and cellular responses in bacterial-induced placental injury and neurobehavioral outcomes in males. Lewis dams were injected daily from gestational day (G) 18 to 21 with corn oil (vehicle) or an androgen receptor antagonist (flutamide). On G 19, dams were injected with saline (control) or GBS. Maternal, fetal sera and placentas were collected for protein assays and in-situ analyses on G 22. In a similar protocol, dams gave birth naturally on G 23 and behavioural tests were performed from post-natal day (P) 9 to 50. Our results showed that while flutamide alone had no effect, a decrease in placental concentration of pro-inflammatory cytokines and infiltration of polymorphonuclear cells was observed in flutamide/infected compared to vehicle/infected groups. Flutamide also alleviated GBS-induced autistic traits of male offspring. These results show that androgens upregulate the placental innate immune response and thus may contribute to the skewed sex ratio towards males observed in several developmental impairments resulting from perinatal infection/inflammation.

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Downregulation of glutamate transporter EAAT1 as a result of endosomal mis-trafficking is involved in Christianson Syndrome ataxia and can be rescued with a modulator of endosomal acidification

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Christianson syndrome (CS) ataxia is a rare X-linked neurodevelopmental and neurodegenerative disorder caused by loss of function mutations in NHE6, an endosomal Na+/H+ exchanger responsible for regulating pH in early and recycling endosomes of the endocytic pathway. Loss of NHE6 results in over acidification of early and recycling endosomes leading to mistrafficking of cargo which could affect neuronal function. In several ataxias, downregulation of glutamate transporters has been reported. Glutamate transporter EAAT1, expressed by Bergmann glia cells in the cerebellum, is critical for preventing glutamate spillover and excitotoxicity at excitatory synapses in the cerebellar cortex. Importantly, elevated glutamate levels have been reported in postmortem analysis of CS patients. Using a dissociated Bergmann glia cell culture and immunofluorescence analysis, we set out to investigate whether the endosomal mis-trafficking of EAAT1 in Bergmann glia cells lacking NHE6 is a mechanism involved in CS ataxia. We observed significant misregulation of EAAT1 in Bergmann glia intracellular trafficking: EAAT1 expression is increased lysosomes, and decreased in early/recycling endosomes. We observed significantly elevated glutamate levels in the cerebella of CS mice, using MALDI-MS imaging. Finally, we show that treatment with a modulator of endosomal acidification, niclosamide, via intraperitoneal injection, can rescue the mis-trafficking of EAAT1 in Bergmann glia cells, leading to partial rescue of PC loss, with potential for therapeutic intervention in CS.

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Structural and biophysical characterization of YopJ Acetyltransferases

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Structural and biophysical characterization of YopJ Acetyltransferases Bacteria use a variety of strategies to evade host immune responses. In gram-negative bacteria, bacterial effector proteins are directly injected into host cells by the type three secretion system, resulting in the modulation of intracellular signaling pathways. My project focuses on a group of such effectors called the YopJ (Yersinia outer protein J) acetyltransferases, which reside in animal pathogens such as Vibrio parahaemolyticus and Salmonella enterica. They use acetyl CoA and the co-factor inositol hexaphosphate (IP6) to acetylate (Ac) host protein kinases on amino acids that normally get phosphorylated. This suppresses host immune signaling, such as the MAPK pathways, allowing the bacteria to replicate in a low inflammatory environment leading to several human illnesses. Hence, a detailed mechanistic and structural understanding of how YopJs target MAPKs will be crucial for therapeutic development. Objectives: 1) Determine the crystal structures of the YopJs in complex with their cognate MKKs to shed light on substrate specificity and catalytic mechanism. 2) Determine the role of autoacetylation in YopJ effectors through mutagenesis, mass spectrometry and activity measurement. Results: 1) Crystals were obtained for VopA-MKK4 (25 aa-linker) and are still undergoing optimization before diffraction analysis. 2) The VopA(S163Ac) crystals diffracted to 2.0 Å and gave insight into substrate, IP6 and CoA binding sites. Moreover, it was found that the autoacetylation sites (Ser163 and Thr200) on VopA inhibit its activity on MKK4 by decreasing Vmax (Ser163) and Km (Thr200) compared to non-autoacetylated VopA. Together, this study is an important step towards the development of therapeutic drugs in autoimmune disorders, and several infectious and inflammatory diseases.

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Therapeutic Exercise Interventions in Rat Models of Arthritis

Hannah Derue, Dr. Alfredo Ribeiro-da-Silva

Arthritis is the leading cause of musculoskeletal pain and disability worldwide. Nearly 50% of individuals over the age of 65 have arthritis, which contributes to limited function, articular pain, physical inactivity, and diminished quality of life. Therapeutic exercise is often recommended in clinical settings for patients experiencing arthritic pain, however, there is little practical guidance regarding the use of therapeutic exercise to alleviate arthritic musculoskeletal pain.

Rodent models of arthritis allow researchers to control experimental variables, which cannot be done with human participants, providing an opportunity to test therapeutic approaches in preclinical models. This literature review provides a summary of published findings in therapeutic exercise interventions in rat models of arthritis as well as gaps in the existing literature. We reveal that preclinical research in this field has yet to adequately investigate the impact of experimental variables in therapeutic exercise including their modality, intensity, duration, and frequency on joint pathophysiology and pain outcomes.

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