PHARMACOLOGY RESEARCH DAY

— 2018 —

INNOVATIVE MEDICINES

MÉDICAMENTS NOVATEURS

CANADA


Pfizer


GE Healthcare


VERTEX™


PEPROTECH®
Manufacturer of Quality Cytokine Products

charles river


ThermoFisher
SCIENTIFIC


MilliPORE
SiGMA

Boehringer Ingelheim

We make it visible.

Special Thanks To All Of Our Sponsors!

Innovative Medicine

GE Healthcare

Pfizer Canada Inc

Vertex

PeproTech

Charles River

ThermoFisher

Millipore Sigma

Boehringer Ingelheim

Carl Zeiss Canada Ltd

Table of Contents

2018 PHARMACOLOGY RESEARCH DAY COMMITTEE

Dr. Bastien Castagner, Chair of Research Day
Dr. Jason Tanny, Co-Chair of Research Day
Bobbi Bidochka
Tina Tremblay
Morgan Foret
Issan Zhang
Han Yan
Anne-Sophie Pepin

A very special Thank You to:

Anna Cuccovia
Chantal Grignon

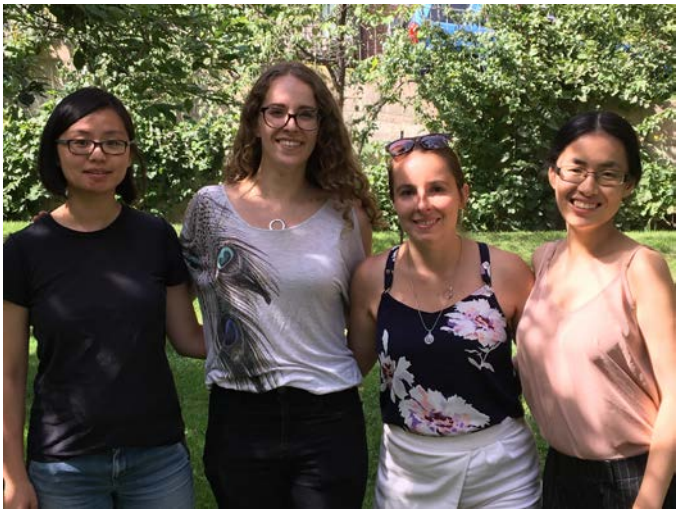
Dear Colleagues,

Welcome to the 24th Annual Pharmacology Research Day!
This year, we are thrilled to welcome our keynote speaker Dr. Pamela Silver, presenting on: *Designing Biology for Living Therapeutics.* We are eager to learn about Dr. Silver's work and expertise on synthetic biology, a rapidly evolving interface between biology and engineering.

We are looking forward to showcase cutting-edge research from students of the Department of Pharmacology and Therapeutics, as well as other associated departments. We hope you will enjoy the talks and posters presented throughout the day and take the opportunity to network and learn about exciting research topics.

We would like to extend special thanks to event sponsors for their generous contributions and members of the organizing committee – Dr. Bastien Castagner (Chair of PRD), Dr. Jason Tanny (Co-Chair of PRD), Dr. Gerhard Multhaup, and Tina Tremblay – for their dedication and hard work in planning and implementing this fantastic event.

Sincerely,
Students of the Pharmacology Research Day Committee



Left to right: Issan Zhang, Morgan Foret, Anne-Sophie Pepin, Aileen Han.

Keynote Speaker

Dr. Pamela Silver

Elliot T and Onie H Adams Professor of Biochemistry
and Systems Biology
Harvard University and Wyss Institute of Biologically
Inspired Engineering

Pamela Silver received her BS in Chemistry and PhD in Biochemistry from the University of California. She was a Postdoctoral Fellow at Harvard University in the department of Biochemistry and Molecular Biology. Pam was an Assistant Professor in the department of Molecular Biology at Princeton University where she was an Established Investigator of the American Heart Association, a Research Scholar of the March of Dimes and was awarded an NSF Presidential Young Investigator Award. Pam moved to the Dana Farber Cancer Institute where she was a Professor in the department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. In 2004, Pam became one of the founding members of the Department of Systems Biology at Harvard Medical School and the first Director of the Harvard University PhD Program in Systems Biology. In 2009, she became one of the first members of the Harvard University Wyss Institute for Biologically Inspired Engineering. In 2012 Pam was named the Elliot T and Onie H Adams Professor of Biochemistry and Systems Biology at Harvard. The Silver Lab works at the interface between systems and synthetic biology to design and build biological systems.

PHARMACOLOGY RESEARCH DAY PRIZES

Melville Prizes

This prize, established in 1994, is awarded annually to the Pharmacology students in both the Junior and Senior category whose research poster presentation at the Pharmacology Research Day is judged to be the best. A Melville Prize is also awarded to a Pharmacology Postdoctoral Fellow whose research poster/oral presentation is judged to be the best.

Best Oral Presentation Prize

This prize is awarded annually to a Pharmacology graduate student for the oral presentation that best exemplifies multidisciplinary approach in Pharmacology.

PRD Life Sciences Prize

(Anatomy and Cell Biology, Biochemistry, IPN)
This prize, established this year by Pharmacology & Therapeutics, is awarded to a graduate student presenting the best posters/orals at Pharmacology Research Day.

Pharmacology Research Day Judges

Dr. Derek Bowie
Dr. Bastien Castagner
Dr. Paul Clarke
Dr. John Gillard
Dr. Terry Hébert
Dr. Gerhard Multhaup
Dr. Lisa Munter
Dr. Alfredo Ribeiro-da-Silva
Dr. Bernard Robaire
Dr. Jason Tanny
Dr. Jean-François Trempe
Dr. Aimee Katen
Dr. Anaïs Noblanc
Dr. Adeola Shobo

24th Annual Pharmacology Research Day

Friday, September 14th, 2018
New Residence Hall
McGill University
3625 Avenue du Parc
Montreal, QC H2X 3P8

PROGRAM

08h00-08h45	**Registration** –Ballroom Lobby **Poster Set-up** – Prince Arthur A *Light breakfast snacks will be provided*
08h50-09h00	**Opening Remarks** – Prince Arthur B Dr. Gerhard Multhaup, Professor and Chair
09h00-09h45	**ORAL SESSION I:** Prince Arthur B Moderators: Morgan Foret & Aileen Yan
09h00	Adamo Mancino, MSc Junior-IPN, lab of Dr. Derek Bowie
09h15	David McCusty, MSc Senior, lab of Dr. Bastien Castagner
09h30	Luc Truong, MSc Junior, lab of Dr. Jean-Francois Trempe
09h45-11h15	**BREAK & POSTER SESSION 1** Odd Numbered Posters. Prince Arthur A
11h15-12h00	**ORAL SESSION II:** Prince Arthur B Moderators: Aileen Yan & Issan Zhang
11h15	Ryan Martin, PhD Senior, lab of Dr. Terry Hebert
11h30	Fiona Hui, MSc Senior, lab of Dr. Timothy Geary
11h45	Jenna Giubilaro, PhD Senior, lab of Dr. Stephane Laporte

12h00-13h30	**LUNCH** – Ballroom Lobby *Please visit the **Sponsors** booths during lunch*
13h30-14h00	**ORAL SESSION III:** Prince Arthur B Moderators: Issan Zhang & Morgan Foret
13h30	Lindsay Welikovitch, PhD Senior-IPN, lab of Dr. Claudio Cuello
13h45	Adelaide Allais, PhD Junior-UdeM, lab of Dr. Greg Fitzharris
14h00	Baraa Noueihed, PhD Senior, lab of Dr. Sylvain Chemtob
14h15-15h45	**BREAK & POSTER SESSION 2** Even Numbered Posters. Prince Arthur A
15h45-17h00	**KEYNOTE ADDRESS:** Prince Arthur B **Dr. Pamela Silver** Elliot T and Onie H Adams Professor of Biochemistry and Systems Biology Member, Harvard University Wyss Institute of Biologically Inspired Engineering ***Title: Designing Biology for Living Therapeutics***
17h00-18h30	**Awards Presentation/Closing Remarks** **Cocktail Reception** - Prince Arthur B

Presenter: Adamo Mancino 09h00 /MSc Jr./O1

Title: Structure-function relationships underpinning brain-specific Nav1.5 voltage-gated sodium channels

Authors: Mancino, Adamo S; Yan, Yuhao; Aurousseau, Mark R.P.; Bowie, Derek;

Abstract: The voltage-gated sodium channel Nav1.5 is studied mainly for its contribution to the cardiac action potential. However, there is growing evidence to suggest that it is also expressed in the brain, where its role remains largely unexplored. Nav1.5 is subject to alternative splicing at exon 6, such that Nav1.5/mH1 retains exon 6b while Nav1.5e acquires exon 6a instead. This alternative splicing may have repercussions on neuronal firing, given that the main difference is a ~10 mV depolarizing shift in the activation profile of Nav1.5e relative to Nav1.5/mH1. The two splice-variants differ at 7 amino acid residues, all of which are located in the voltage sensor domain (VSD) of domain I. Even though these residue exchanges occur in a part of the channel which is critical for pore opening, their specific structure-function relationships are not fully explored. Here, we engineered single point mutations, introducing key amino acids from Nav1.5e into Nav1.5/mH1 and vice versa, and identified that two important amino acid switches, aspartate-lysine at position 211 and threonine-serine at position 207, are responsible for the altered gating profile. We speculate that these exchanges alter the network of counter-charges in the domain I VSD as a mechanism of action. Furthermore, in order to examine the relative contribution of each domain to the activation process, we neutralized the gating charges across each domain and noticed that domain I VSD is the major determinant, lending insight into why splicing evolved in domain I. This study illustrates the mechanism by which alternative splicing in domain I modulates the functional properties of Nav1.5, which may in turn contribute to the fine-tuning of cell excitability.

Acknowledgments: Special thanks to authors, and funding sources, NSERC and CIHR.

Program

Presenter: David McCusty 09h15/MSc Sr./O2

Title: Bone-targeted Fluorogenic Probe for the Functional Imaging of Mineralized Tissues

Authors: David McCusty, Reem Kurdieh, Emmanuelle LeBlanc, Svetlana Komarova, Bastien Castagner

---

Abstract: Fluorescent probes are widely to investigate biological processes. A major limitation in their use, however, is the delivery of the probe to the location of interest and maintaining the probe in that location long enough to be able to detect a useful fluorescence signal. To mitigate this issue, a fluorescent probe conjugated to the bone-targeting drug alendronate via a maleimide linker has been synthesized and successfully purified, which facilitates probe binding to the inorganic hydroxyapatite mineral in bone tissue. By using a Förster resonance energy transfer (FRET) internally quenched peptide, the probe is also made stimulus responsive. Upon enzymatic cleavage of the peptide by the protease Cathepsin K, a quencher is separated from a fluorescent dye, resulting in an increase of fluorescence, which is measured in a fluorescence plate reader or imaged using fluorescent microscopy. Cathepsin K is an important protease, as it is the major protease expressed by osteoclasts, the only cell capable of resorbing bone tissue, and it is largely responsible for the break down of the organic collagen tissue in bone. This probe is designed to not only indicate the presence of cathepsin K, but it is also capable of detecting its activity, important for proteases like Cathepsin K with both active and inactive forms. Furthermore, the probe is also a proof-of principle of a bone-targeting probe that can be applied to other proteases; by changing the amino acid sequence of the peptide, different proteases could be investigated using a similar design.

Acknowledgments: Dr. Mark Hancock, Dr. Gulzhakhan Sadvakassova, Zamboni Chemical Solutions, NSERC

Program

Presenter: Luc Truong 09h30 /MSc Jr./O3

Title: Paradoxical agonists as PINK1 activators for Parkinson's disease

Authors: Truong, Luc; Trempe, Jean-François

Abstract: Early-onset Parkinson's disease (PD) is characterized by the preferential loss of dopaminergic neurons in the substantia nigra. This targeted neurodegeneration has been linked to an incompetence of the mitochondrial quality control pathway (MQC) via loss-of-function mutations in PINK1, a ubiquitin (Ub) kinase, and Parkin, an E3 Ub ligase. Normally, PINK1 selectively accumulates on depolarized mitochondria and phosphorylates nearby Ub as well as the ubiquitin-like domain (Ubl) of Parkin. This leads to Parkin activation and subsequent ubiquitination of substrates on the outer mitochondrial membrane, thus providing more substrate for PINK1 to phosphorylate: a positive feedback loop is therefore established. Eventually, phospho-ubiquitin decoration of the mitochondria marks it for isolation, repair, or mitophagy. The dysfunction of Parkin or PINK1 causes accumulation of toxic, damaged mitochondria, ultimately causing cell death. This pathway could be exploited to provide a much-needed disease-modifying therapy for PD. Newly published structures of PINK1 reveal its only small-molecule binding site to be its ATP binding site. Though ATP-competitive small molecules are designed to inhibit kinases, recent evidence shows that in some cases, ATP-competitive inhibitor binding results in kinase oligomerization, hyperactivation of partially inhibited kinase dimers, or increased kinase localization to subcellular destinations. The small-molecule catalytic inhibition is thus compensated for by these structural rearrangements in what is called "paradoxical activation." As a readout for structural rearrangement, we sought for paradoxical PINK1 activators by screening kinase inhibitors for their ability to confer thermal stability by a thermal shift assay. Of the top hits, CYC116, an Aurora kinase inhibitor, was shown to inhibit insect PINK1 substrate phosphorylation, autophosphorylation, and ATP hydrolysis, but did not affect human PINK1 activity on extracted mitochondria.

Acknowledgments: Nathalie Croteau, Shafqat Rasool, other members of the Trempe Lab; GEPROM, NSERC, FRQS, McGill University

Program

Presenter: Ryan Martin 11h15 /PhD Sr./O4

Title: Signal-specific function of P-TEFb complexes in the development of cardiac hypertrophy

Authors: Martin, Ryan; Sun, Yalin; Tanny, Jason; Hébert, Terence

---

Abstract: Heart disease is characterized by remodelling of the cardiac tissue in response to chronic mechanical and hormonal stress placed upon the heart. Initial remodelling events preserve cardiac function through hypertrophy of the heart's contractile cardiomyocytes but is ultimately maladaptive and can lead to heart failure. A potential therapeutic target currently being explored is recruitment of the transcription regulator positive transcription elongation factor b (P-TEFb). Hormonal activation of different GPCRs implicated in cardiac remodelling, such as the α1-adrenergic receptor (α1AR) or endothelin receptor (ETR), leads to an increase in P-TEFb activity and chromatin recruitment in cardiomyocytes. P-TEFb is recruited as a constituent of two distinct complexes, one with the bromodomain and extra-terminal protein Brd4 and the other with the Super Elongation complex (SEC). How signalling downstream of distinct GPCRs leads to activation of either complex and subsequent hypertrophic phenotype in cardiomyocytes, and how this effects their therapeutic potential, is not known.
We hypothesized signaling through distinct GPCRs elicits similar morphological and gene expression programs through distinct activation of different P-TEFb complexes. Transcriptome analysis demonstrated that α1AR or ETR activation in primary neonatal rat cardiomyocytes initiated similar gene expression programs and hypertrophic phenotype. Although P-TEFb activity was required downstream of both receptors, the recruitment mechanism differed. The SEC was required for both responses; however, Brd4-mediated recruitment was only required for the α1AR-mediated hypertrophic response. We are investigating a novel mechanism of Brd4 activation and recruitment through α1AR-mediated activation of protein kinase A. The role of the Brd4/P-TEFb complex in response to selective GPCR activation has potential clinical implications as therapies targeting this complex are currently being explored for heart failure.

Acknowledgments: This work was supported by the Heart and Stroke Foundation of Canada and the Canadian Institutes of Health Research (CIHR).

Program

Presenter: Fiona Hui 11h30 /MSc Sr./O5

Title: Characterization of neuropeptide GPCRs NPR-4 and NPR-5 from the parasite Brugia malayi

Authors: Hui, F.; Geary, T.

Abstract: In recent years, the neuropeptidergic system has become a target for anthelmintic discovery, due to its essential roles in nematodes. Deorphanization of neuropeptide GPCRs that recognize members of the invertebrate-restricted FMRFamide family has been extensively carried out in the free-living model organism Caenorhabditis elegans. Two of these receptors, NPR-4 and NPR-5, were shown to be activated by FMRFamide-like peptides (FLPs) encoded on the flp-18 precursor gene. However, the relevance of NPR-4 and -5 in a parasitic nematode context has yet to be investigated. In this study, we characterized Bma-NPR-4 and Bma-NPR-5 from the parasitic nematode Brugia malayi. We hypothesize that Bma-NPR-4 and -5 can be activated by FLP-18 peptides with activity profiles comparable to those of the C. elegans orthologues. Bma-NPR-4 and -5 were heterologously expressed in both Chinese hamster ovary (CHO) cells and yeast (Saccharomyces cerevisiae). The extent of GPCR activation was quantified using an aequorin-based Ca2+ bioluminescence assay in CHO cells and an Alamar Blue-based yeast proliferation assay. Thirteen C. elegans FLP-18 peptides activated Bma-NPR-4 and -5 expressed in CHO cells. The efficacy of FLP-18 peptides are comparable, with a higher potency against Bma-NPR-4 than -5. In yeast, Bma-NPR-4 was functionally expressed but showed more variation in peptide potency and efficacy, while we were not able to functionally express Bma-NPR-5 in this system. Structure-activity relationships (SAR) of Bma-NPR-4/-5 were further investigated using truncated analogues and an alanine scan series of FLP-18f (DVPGVLRFa), The essential role of the -VLRFa motif in receptor activation was demonstrated for both receptors. SAR profiles of Bma-NPR-4 and -5 were generally conserved with slight differences, and were comparable to NPR-4 and -5 orthologues from C. elegans. The insights gained can contribute to future efforts to discover non-peptide ligands as anthelmintics.

Acknowledgments: This research was funded by NSERC and the Canada Research Chair program awarded to Dr. Timothy Geary. Heterologous expression in CHO cells was done in collaboration with the Functional Genomics and Proteomics
Research Group of KU Leuven.

Program

Presenter: Jenna Giubilaro 11h45 /PhD Sr/O6

Title: Identification of a Novel Inhibitor of Small G Proteins: Arf6 and Ras

Authors: Giubilaro, J.; Namkung, Y.; Laporte, S. A.

Abstract: G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors and the target of 40% of therapeutic drugs. Activated GPCRs can signal via a G protein- or via a β-arrestin-dependent mechanism. β-arrestins are adaptor proteins that desensitize G protein signaling, promote receptor internalization, and activate signal transduction cascades, such as mitogen-activated protein kinases (MAPK). The spatiotemporal aspect of these pathways makes it difficult to study the various roles β-arrestins play in regulating GPCRs and complicates the development of effective therapeutics targeting these receptors. Therefore, we performed a high throughput screen in HEK293 cells and identified trafficking molecule #21 (Traf 21) as an inhibitor of angiotensin II type 1 receptor (AT1R) internalization, which is further characterized here.

Using BRET-based sensors, we show that Traf 21 affects β-arrestin recruitment to AP-2 in clathrin-coated pits (CCPs) but not to AT1R. This lead us to investigate other proteins involved in the formation of CCPs, such as the small G protein Arf6. We developed a new set of BRET-based sensors and used GST pulldown to show that Traf 21 decreases the activation of Arf6. Since receptor internalization can mediate β-arrestin signaling, it is not surprising that Traf 21 also inhibits receptor-mediated MAPK signaling. However, this inhibition is due to its effects on the small G protein Ras, which plays a role in this pathway. Furthermore, looking at other members of this small G protein family, Traf 21 does not affect the activation of Rac and Rho.

In conclusion, Traf 21 may independently affect Arf6 and Ras, or their respective downstream pathways may be linked. Investigating how Traf 21 can inhibit both small GTPases may lead to a better understanding of the mechanisms regulating GPCRs and help to develop better therapeutics targeting these receptors.

Acknowledgments: This work was supported by the CIHR and the RQRM.

Program

Presenter: Lindsay Welikovitch 13h30 /PhD Sr-IPN/O7

Title: The neuron-inflammatory effects of intracellular Aβ: implications for the development of early Alzheimer's disease

Authors: Welikovitch, Lindsay; Do Carmo, Sonia; Cuello, A. Claudio

Abstract: Inflammation in Alzheimer's disease (AD) has classically been considered a glial and plaque driven phenomenon: activated microglia become coordinately recruited towards Aβ plaques, releasing toxic inflammatory molecules and inciting local neuronal damage. We now understand that the AD neuropathology develops decades before symptom onset, and chronic NSAID use during this asymptomatic period curtails disease development. It has also been shown in transgenic animal models that neuroinflammation begins before plaque deposition, coincident with significant cognitive deficits and intraneuronal Aβ accumulation.

Here, we explore a potential role for the Aβ-burdened neuron as a primary inflammatory agent in the earliest stages of the Aβ pathology, when Aβ accumulation is restricted to the intraneuronal space.

To evaluate the inflammatory gene expression profile of Aβ-burdened neurons, we used laser capture microdissection to excise neurons from the hippocampus of WT and pre-plaque McGill-R-Thy1-APP rats and subjected them to qRT-PCR. Next, we measured the neuronal expression of inflammatory molecules by performing neuron-specific fluorescence quantification following immunolabeling. We then used RNA fluorescent in situ hybridization to demonstrate the cell-specific localization of inflammatory transcripts.

We show for the first time that Aβ-burdened neurons initiate a potent inflammatory signal driven by an upregulation in MCP-1, MIP-1α, and IL-6 before Aβ plaque deposition. We also show that, coincident with the neuronal production of inflammatory factors, activated microglia become recruited towards Aβ-burdened neurons, but do not appear to contribute to the neuroinflammatory environment. While low levels of intraneuronal Aβ can be detected in non-cognitively impaired individuals, our findings suggest that abnormally high intraneuronal Aβ accumulation unleashes an early pro-inflammatory response, laying the groundwork for the AD neuropathological cascade.

Acknowledgments: We would like to thank the McGill University Advanced BioImaging Facility (ABIF) for their help with confocal microscopy.

Program

Presenter: Adelaide Allais 13h45/PhD Jr-UdeM/O8

Title: Determining the impact of mitosis length on early embryo health

Authors: Allais, A.; FitzHarris, G.

Abstract: Preimplantation embryos often comprise cells with the correct (euploid) and wrong (aneuploid) number of chromosomes due to chromosome segregation errors. Such ‘mosaic’ embryos may reduce reproductive success, but how these errors arise remains unclear. The timing of early embryo cell divisions is highly variable in embryos, and is emerging as a potential indicator of embryo health in fertility clinics (termed ‘morphokinetics'). In somatic cells, extended mitosis can cause premature separation of sister chromatids, a phenomenon known as ‘cohesion fatigue’, which could cause aneuploidy. Thus, we set out to determine whether embryos are susceptible to cohesion fatigue. We manipulated the duration of mitosis in two-cell stage embryos with the APC inhibitor APCin. Live 3D confocal imaging of Sir-Tubulin, H2B:RFP and MajSatTALE:mClover was used to monitor spindle, chromatin, and pericentromeric regions, respectively. This revealed that mitotic arrest triggered a pronounced spindle elongation and loss of chromosome alignment. Fixed cell analysis confirmed a time dependent loss of chromosome alignment and revealed that almost all misaligned chromosomes (97%) were prematurely separated sister chromatids. The loss of sister cohesion was preceded by an increase in inter kinetochore distances, consistent with cohesion fatigue. Formal counting of the proportion of separated sisters by labelling kinetochores after spindle disassembly revealed that 4% of all sister pairs had individualised by 6 hours of mitotic arrest, and 66% by 24 hours. Strikingly, ongoing experiments suggest that embryos with this extreme level of cohesion fatigue can nonetheless continue to divide in culture. Therefore, our data revealed that when faced with prolonged mitosis, the early embryo is susceptible to cohesion fatigue and this type of genetic error may in fact not prevent development. We thus speculate that cohesion fatigue may be an underlooked cause of aneuploidy in the preimplantation embryo.

Acknowledgments: Funding Sources: Supported by a Canadian Institutes of Health Research (CIHR) Operating Grant.

Program

Presenter: Baraa Noueihed 14h00/PhD Sr./O9

Title: Mesenchymal stromal cells (MSCs) promote vascular repair in Ischemic Retinopathies by modulating inflammation

Authors: Noueihed, Baraa; Rivera, Jose Carlos; Chemtob, Sylvain

---

Abstract: Purpose: Ischemic retinopathies are characterized by vaso-obliteration (VO) of the retinal vascular network, resulting in tissue hypoxia, followed by pathological neovascularization (NV). Current treatment modalities slow down the exaggerated growth of aberrant vessels; however, they do not regenerate the damaged vascular bed and could cause adverse side effects. Mesenchymal stromal cells (MSCs) serve as an alternative therapeutic approach due to their pro-angiogenic and anti-inflammatory properties. We hypothesize that MSCs can promote vascular repair by modulating inflammation in ischemic retinas.
Methods: Oxygen-induced retinopathy (OIR) mouse model which mimics the hallmarks of ischemic retinopathies was used herein. Postnatal day 7 (P7) mice were subjected to 75% O2 until P12 to induce VO followed by 5 days of room air leading to NV. MSCs were isolated from the compact bone of adult mice and cultured in hypoxia. Supernatants (CM) were collected 24 hours later and injected intravitreally at P12. VO and NV areas were assessed at P17. To determine the effect of CM on inflammation in treated retinas, mRNA levels of various inflammatory mediators were measured by quantitative PCR. In vitro, primary bone-marrow macrophages (BMDM) were polarized into M1 pro-inflammatory phenotype, and subsequently treated with CM. Expression levels of pro-inflammatory cytokines were quantified by qPCR.
Results: Treatment of OIR retinas with CM significantly reduced VO and NV areas at P17 in comparison to vehicle. Gene expression levels of the pro-inflammatory mediators (IL-1β, TNFα, iNOS) were decreased, where as the anti-inflammatory cytokines (IL-10, IL-4) were increased in CM-treated retinas. Exposure of M1-BMDM to CM significantly reduced its pro-inflammatory profile.
Conclusions: MSC-CM repopulated the vaso-obliterated areas and inhibited aberrant neovascularization in OIR retinas. This effect is mediated in a paracrine fashion by curtailing the pro-inflammatory response.

Acknowledgments: FRQS

Program

POSTER SESSION I
09h45 – 11h15

P1	Emilie Brule	PhD Junior	Anatomy
P3	Erik Larson	PhD Senior	IPN
P5	Fernando Altamura	MSc Junior	Pharmacology
P7	Celia Bouazza	MSc Junior	Pharmacology
P9	Anthony Duchesne	MSc Junior	Pharmacology
P11	Wanru Guo	MSc Junior	Pharmacology
P13	Iulia Pirvulescu	MSc Junior	Pharmacology
P15	James Eng	MSc Senior	Pharmacology
P17	Xiao Ming (Sindy) Zheng	MSc Senior	Pharmacology
P19	Mozhdeh Mehdizadeh	PhD Junior	Pharmacology
P21	Xiaotong Wang	PhD Junior	Pharmacology
P23	Kyla Bourque	PhD Senior	Pharmacology
P25	Colin Cheng	PhD Senior	Pharmacology
P27	Sean Jmaeff	PhD Senior	Pharmacology
P29	Gauthier Schang	PhD Senior	Pharmacology
P31	Issan Zhang	PhD Senior	Pharmacology

Presenter: Emilie Brûlé 09h45/ PhD Jr-ANAT/P1

Title: IGSF1 does not regulate FSH synthesis or secretion in vivo or in vitro

Authors: Brûlé, Emilie; Li, Yining; Schang, Gauthier; Wang, Ying; Bernard, Daniel J.

Abstract: Loss of function mutations in the X-linked immunoglobulin superfamily, member 1 (IGSF1) gene result in central hypothyroidism, often associated with macroorchidism. Igsf1 knockout mice are also centrally hypothyroid, due to impaired thyrotropin-releasing hormone (TRH) receptor expression and TRH action in the pituitary. The mechanisms underlying testicular enlargement are unclear and disputed. IGSF1 was originally characterized as an inhibin co-receptor. As inhibins negatively regulate follicle-stimulating hormone (FSH) secretion, it was hypothesized loss of IGSF1 would lead to impaired inhibin action, elevated FSH, and, as a result, enhanced Sertoli cell proliferation during post-natal development. However, IGSF1 does not associate with inhibin A or B in heterologous binding assays. More recently, IGSF1 was proposed to inhibit signaling by the activin type IB receptor (ALK4). As activins stimulate FSH, the loss of this inhibition should lead to enhanced FSH levels. However, neither humans nor mice with IGSF1-deficiency have elevated FSH. Moreover, the methods used to demonstrate IGSF1 regulation of human FSHB promoter-reporter were conducted in a heterologous assay system in which such reporters lack activin/ALK4-dependent activity. Here, we further demonstrate that, when over-expressed in a homologous cell system (LβT2 cells), IGSF1 does not impair induction of murine or human Fshb/FSHB promoter-reporters by activin A or a constitutively active form of ALK4. Preliminary data further indicate that Fshb mRNA expression is similarly antagonized by inhibin A and B in primary cultures of pituitaries from wild-type and Igsf1-deficient mice. Collectively, the available data fail to support a role for IGSF1 in FSH regulation by activins, inhibins, or otherwise.

Acknowledgments: This research was supported by CIHR operating grant MOP-133557 to DJB and NSERC CGS-D training grant awarded to EB.

Odd Posters

Presenter: **Erik Larson** 09h45/PhD Sr-IPN/P3

Title: NMDA receptor elevation of cytosolic reactive oxygen species strengthens GABAergic signaling

Authors: Accardi, Michael V.; Wang, Ying; D'Antoni, Martina; Karimi, Benyamin; Siddiqui, Tabrez J; Bowie, Derek

---

Abstract: In recent years there have been new advances towards understanding the nature of inhibitory signaling in the brain. Previous work has shown a novel mechanism for strengthening of inhibitory GABAergic signaling by cytosolic reactive oxygen species (ROS) elevated by insulin signalling. Whether GABAergic synapses can also be potentiated by ROS generated by excitatory neurotransmission has yet to be examined. To investigate this question, we performed whole-cell electrophysiological recordings from molecular layer interneurons of the mouse cerebellum. Additionally, we placed an extracellular stimulating electrode in the molecular layer of the cerebellum to activate a network of excitatory parallel fibers (PFs) from granule cells and neighbouring inhibitory interneurons. As anticipated, high frequency activation stimulation of PFs activated extrasynaptic NMDARs which led to elevated cytosolic ROS. This in turn caused a time-dependent increase in the strength of GABAergic synapses. The use of pharmacological blockers suggest that the origin of ROS generated by NMDAR activation is due to the combined activities of neuronal nitric oxide synthase and neuronal NADPH oxidase. Taken together, our data reveal a novel mechanism for the strengthening of GABAergic transmission through a NMDAR-ROS mediated pathway.

Acknowledgments: CIHR, Savoy Foundation

Odd Posters

Presenter: Fernando Altamura 09h45/ MSc Jr./P5

Title: Revealing gut microbiota metabolism with dietary glycan-fluorophore conjugates

Authors: Altamura, F.; Dridi, L.; Kubinski, R.; Castagner, B.

Abstract: Background: The human gastrointestinal tract hosts almost a trillion microorganisms, organized in a complex ecosystem known as the microbiota. Our bacterial genetic diversity dictates the digestion of complex dietary carbohydrates (glycans). Indeed, the human genome only encodes a few carbohydrate-active enzymes (CAZymes) that are able to metabolize complex sugars found in conventional food sources such as vegetables, plants, and grains. Consequently, most of these glycans reach the large intestine fully intact, where they are digested by the gut microbiota, which encodes a much wider repertoire of CAZymes. Even if we know that diet dramatically impacts our gut microbiota, we need a functional and molecular understanding of how dietary glycans shift this complex ecosystem.
Aims: To link specific glycan structures to the metabolizing gut bacteria, and to understand how the composition of the overall ecosystem modulates and adapts after the administration of meals that are rich in those glycans.
Methods: (1): Synthesis of fluorescent probes. A library of dietary glycans conjugated to a fluorescent dye will be synthetized and purified. (2): Uptake parameters optimization. Biochemical and kinetic parameters for the uptake of the synthetized glycans in different representative gut bacterial isolates will be optimized in vitro. (3): Identification of glycan consumers in the human microbiota. Using stool samples as proxies for the gut microbiota, the bacterial species consuming our probes will be sorted by Fluorescence-Activated Cell Sorting (FACS) and identified with 16S RNA sequencing.
Obtained Results: We successfully synthesized ten probes, optimized their purification protocols using liquid chromatography, and characterized their structures using NMR and Mass Spectrometry. We also validated the specific metabolic labeling of a few bacterial isolates, and characterized the labeling by fluorescence microscopy and flow cytometry.

Acknowledgments:

Odd Posters

Presenter: Célia Bouazza 09h45/ MSc Jr./P7

Title: Distinct functions of Gβγ isoforms in transcriptional regulation

Authors: Célia Bouazza; Darlaine Pétrin; Terry Hébert

Abstract: Communication between cardiomyocytes and fibroblasts is strongly implicated in cardiac disorders, however the mechanisms of intercellular interplay by which cardiac hypertrophy and fibrosis are regulated remain unclear. In order to define new therapeutic targets, it is essential to identify links between fibroblasts activation by the G protein-coupled type 1 receptor for angiotensin II and intracellular signaling leading to fibrotic gene transcription. This project will explore the impact of isoforms of the Gβγ subunit of heterotrimeric G proteins on transcription in HEK 293 cells and in rat neonatal cardiac fibroblasts (RNCFs) to understand their unique roles in activating fibrotic signaling cascades. Our lab has shown that Gβγ dimers are found at over 700 promoters in HEK 293 cells. This led us to identify an angiotensin II-mediated interaction between Gβγ and RNAP II. To better understand the interaction, we studied the silencing of specific Gβγ isoforms on expression of fibrotic genes. Our results suggest that Gβ1 acts as a regulator of gene expression and that its absence dysregulates the fibrotic response. This project focuses on applying a proteomic screen to identify the interacting partners of Gβγ isoforms at various stages in the transcription of individual genes. First, we will set up a screen to study specific gene loci under control conditions and following carbachol-stimulation of endogenous M3-mAChR in HEK 293 cells. Following this, we will apply it in RNCFs to study the proteomes at specific gene loci during the fibrotic response to Ang II and see how and when Gβγ dimers are recruited to these sites. We will use a technique called caspex which allows targeting of specific DNA sequences by guide RNA and labeling of nearby proteins with biotin. Once labeled, they can be identified by mass spectrometry. Our results will establish a link between particular Gβγ isoforms and fibrotic gene regulation through the generation of dimer-specific interactomes.

Acknowledgments: The project is funded by CIHR and the Heart and Stroke Foundation.

Odd Posters

Presenter: **Anthony Duchesne** 09h45/ MSc Jr./P9

Title: Measuring Parkison's Disease Mitochondrial Protein Turnover Rates in Human IPSC-Derived Organoids by Mass Spectrometry

Authors: Duchesne, Anthony; Mohamed, Nguyen-Vi; Yi, Wei; Trempe, Jean-Francois

---

Abstract: Parkinson's Disease (PD) is a currently incurable neurodegenerative disorder that manifests in the elderly through motor symptoms of bradykinesia, rigidity and tremor. PD causes a dopamine deficit, which leads to ineffective neural motor function. For unknown reasons, certain neuronal populations involved in the disease will die whilst others nearby that are very similar the same will survive. One of the prevalent theories explaining this selective death is the mitochondria stress hypothesis, where the neuron populations associated with PD are under more stress than others, resulting in their death from mitochondrial quality control mechanisms. Therefore, understanding the mechanisms of the mitochondrial quality control in these PD-associated neural populations is critical. There are two PD-associated proteins: PINK1, a mitochondrial targeted kinase, and Parkin, a ubiquitin ligase. Previous studies have found that the regulation of mitochondrial proteins in fruit flies is impaired by mutations in Parkin and PINK1, characterized by a deficit in mitochondrial protein turnover. Whether mitochondrial proteins are similarly regulated in mammals has yet to be confirmed. In the current study, our aim was to validate the human induced pluripotent stem cell organoids model in measuring protein turnover. We used mass spectrometry proteomics to examine the effect of a Parkin deletion mutation in a human induced pluripotent stem cell (IPSC) organoid model. We used stable isotope labelling in the amino acid leucine to measure protein turnover in time course experiments. Our preliminary results have shown the incorporation of heavy isotope labels into the organoids and a decrease in protein turnover in Parkin mutated organoids. Additional experiments to optimize protein coverage and reliably identify mitochondrial proteins will be conducted. In the future, we will expand to other PD-related organoid models and test drugs that stimulate mitochondrial quality control.

Acknowledgments: I'd like to thank the following individuals for their contributions to my project. My Supervisor: Jean-Francois Trempe.
Lab Members: Andrew Bayne, Luc Truong, Sophie Lu, Nimra Khan, Natalie Croteau and Shafqat Rasool. MNI Collaborators: Nguyen-Vi Mohamed and Wei Yi. Graduate Committee: Lisa Münter, Heidi McBride and Terry Hebert.
And finally the funding agencies: CIHR, HBHL, Michael J. Fox Foundation and Parkinson Canada.

Odd Posters

Presenter: Wanru Guo 09h45/MSc Jr./P11

Title: The effect of polyinosinic-polycytidylic acid on the antibacterial responses to pseudomonas aeruginosa in bronchial epithelial cells

Authors: Guo W., Rousseau S.

Abstract: Viral infections often cause pulmonary exacerbations in cystic fibrosis (CF) patients, and this is seen in patients who are pre-colonized with bacterial pathogens such as Pseudomonas aeruginosa. These co-infections can result in significant decrease in lung function over time. On the other hand, secondary bacterial infections most often occur in the context of pre-existing viral infections. Recently, the Rousseau lab has demonstrated an intracellular reservoir of P. aeruginosa inside epithelial cells. While a diminished antiviral response and increased viral load with co-infection by P. aeruginosa has been shown, we sought to investigate how viral infections affect Pseudomonas intracellular persistence and host antibacterial response. Poly(I:C), a dsRNA that activates Toll-like receptor (TLR) 3, was used to mimic viral infections. A consistently higher p38 MAP kinase activation was seen at various timepoints after transfection of poly (I:C) dsRNA into BEAS-2B cell line compared to stimulation by extracellular poly(I:C), corresponding to its activation of intracellular targets. An additive increase in p38 MAP kinase phosphorylation was seen along with stimulation by Pseudomonas flagellin after transfection of poly(I:C) into BEAS-2B cells. Preliminary data also demonstrated a consistent decrease in internalisation of P. aeruginosa in BEAS-2B cells pretreated with poly (I:C). Overall, these experiments enhance our understanding of how epithelial cells respond to secondary bacterial infection after pre-existing viral infection, and provide an insight into the pathophysiology of pulmonary exacerbations seen in CF patients.

Acknowledgments: CIHR, Cystic Fibrosis Canada

Odd Posters

Presenter: Iulia Pirvulescu 09h45/MSc Jr./P13

Title: A functional study of somatic and germline GNB1 mutations involved in leukemias and neurodevelopmental disorders.

Authors: Pirvulescu, Iulia; Khan, Shahriar; Sleno, Rory; Pétrin, Darlaine; Zhu, Xinwen; Hébert, Terry

---

Abstract: G protein-coupled receptors (GPCRs) represent the largest class of membrane receptors in eukaryotes. Activation of GPCRs by extracellular signals leads activation of associated G protein heterotrimers, composed of alpha, beta and gamma subunits. Mutations in different G proteins have been implicated in a number of different cancers and neurodevelopmental disorders. We focus on a set of eleven different point mutations in the Gb1 subunit, studying the effects of these mutations in vitro, where we may be able to attribute functions to specific Gb1 residues. To study whether mutants can rescue knockdown of WT Gb1, we expressed Flag-tagged, siRNA-resistant copies of
the mutated genes.
Research in the lab has shown that Gb1 regulates M3-mAChR-mediated calcium release in HEK 293 cells; knockdown of the Gb1 subunit leads to a significant increase in intracellular calcium release in response to carbachol treatment. To test whether expression of siRNA-resistant Gb1 could rescue the effect of Gb1 knockdown, we used a luminescence-based functional assay measuring intracellular calcium levels performed in live cells. We show that expression of siRNA-resistant WT Gb1 rescued the increase in calcium release whereas K78E and K89E exhibited dominant negative behavior. Alternatively, mutations A11V, A92T, M101V, D118G caused by Gb1 knockdown. Next, we aimed to determine whether overexpressing the different mutants could also rescue this effect. We show that mutations K57E, D76G and I80T resulted in a loss of function, and I269T were able to rescue the effect of Gb1 knockdown on transducing M3-mAChR-mediated calcium release. Further studies are underway to understand the impact of the mutations on other Gb1-mediated effects.

Acknowledgments: This work was supported by grants for NSERC, CIHR and HSFC to TEH. SK and RS were supported by scholarships from the CIHR McGill Drug Discovery Training Program. XZ was supported by a studentship from NSERC.

Presenter: James Eng 09h45/MSc Sr./P15

Title: Clusterin-amyloid interactions and their role in Alzheimer's disease

Authors: Eng, J.; Multhaup, G.

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory loss and a decline in cognitive function. A critical event in the pathogenesis of AD is the accumulation of amyloid-beta (Aβ) peptides into toxic Aβ oligomers in the brain causing neuronal damage and synaptic loss. Clusterin is an extracellular chaperone protein linked to sporadic AD through genome-wide association studies and clusterin has been previously shown to interact directly with Aβ peptides and modulate their aggregation behavior in vitro. Additionally, increased clusterin levels were found in post mortem brains of AD patients, further suggesting the importance of clusterin in AD. While clusterin is capable of directly binding to Aβ peptides, the mechanism and biological functions of clusterin-Aβ interactions have remained unclear. The present study investigates clusterin-Aβ interactions and the influence of clusterin on Aβ peptide abundance. It was found that, in the presence of clusterin, there was an increase in Aβ40 in conditioned medium, whereas the levels of Aβ42 and Aβ34 showed no change. When clusterin and APP were expressed at different ratios, higher levels of Aβ40 were seen when the clusterin to APP ratio was the highest; however, no changes in Aβ42 or Aβ34 levels were observed. These data suggest that clusterin preferentially affects Aβ40 levels over Aβ42 or Aβ34. Increased Aβ40 levels compared to control may be a result of clusterin binding to Aβ40¬ preferentially, and preventing its interaction with other Aβ species and/ or inhibiting degradation by forming a stable, long-lived complex. Another possible explanation could be an effect of clusterin on the γ-secretase complex which results in an increase of Aβ40 production over other Aβ species.

Acknowledgments: Thank you to Dr. Mark Wilson (University of Wollongong) and to Dr. Mark Hancock (McGill SPR/MS Facility)

Odd Posters

Presenter: Sindy Zheng 09h45/MSc Sr./P17

Title: Exploring the conservation of Gβγ signalling from an evolutionary perspective

Authors: Zheng, Xiao Ming (Sindy); Khan, Shahriar; Sleno, Rory; Devost, Dominic; Trieu, Phan; Hébert, Terry

---

Abstract: Heterotrimeric G proteins, comprised of α, β, and γ subunits, relay signals from G protein-coupled receptors (GPCR) to downstream effectors. Gβγ dimers have been found at multiple subcellular sites serving both canonical and novel non-canonical roles. Our previous phylogenetic analyses of the 5 mammalian Gβ and the 12 mammalian Gγ subunits revealed that Gβ and Gγ subunits diverged into two and five phylogenetically related types, respectively. Of interest to this project, the invertebrate C. elegans, unlike mammals, possesses only two Gβ (GPB1 and GPB2) and two Gγ (GPC1 and GPC2). This difference in Gβ and Gγ subunit number between species led us to consider whether new functions are associated with the expanded diversity in mammals. We hypothesize that mammalian Gβ proteins have conserved ancestral properties in C. elegans but have also evolved novel functions.
Aim 1: Find mammalian signalling pathways sensitive to Gβ protein expression using short interfering RNA (siRNA) in combination with biosensor-based assays.
Aim 2: Investigate how C. elegans Gβ homologs affect mammalian cell signalling pathways identified in Aim 1 by replacing mammalian Gβ subunits with C. elegans Gβ homologs.
Briefly, endogenous M3-mAChR mediated calcium signalling was the first identified Gβ1 and Gβ4 sensitive pathway in HEK 293 cells. Concurrent expression of GPB1 led to the rescue of Gβ4 knockdown resulted decrease in calcium release. cAMP signalling mediated by endogenous β-adrenergic receptors was the second examined pathway; we saw no effects of knocking down Gβ1 or Gβ4 in HEK 293 cells. Therefore, we will examine other downstream signalling pathways such as Rho activation and β-arrestin recruitment with biosensors.

Acknowledgments: This work was supported by grants for NSERC, CIHR and HSFC to TEH. SK and RS were supported by scholarships from the CIHR McGill Drug Discovery Training Program.

Odd Posters

Presenter: Mehdizadeh, Mozhdeh 09h45/PhD Jr./P19

Title: Polymeric nanoparticles designed to achieve targeted drug delivery to cardiomyocytes

Authors: Mehdizadeh, Mozhdeh; Chatenet, David; Naud, Patrice;Nattel, Stanley

---

Abstract: Background: Cardiovascular diseases are a major health problem in the developed. Current available therapies for these conditions have many limitations, including adverse effects and insufficient efficacy. Therefore, finding novel therapeutic approaches for these diseases is urgently required. A promising strategy is targeted drug delivery to heart tissue that provides specific accumulation of therapeutic agents in cardiac cells, with minimal off-target effects. A novel approach would be to design actively targeted Nanoparticles (NPs) with a targeting agent that can accumulate specifically in the cardiac tissue. A cardiac-targeting peptide (CTP) has been described, consisting of a specific 12 amino acid sequence reported to be able to target cardiomyocytes in previous studies. Thus, using this peptide as a targeting agent for polymeric NPs can provide an effective way for designing a novel drug delivery system to heart.
Methods and Results: 1) CTP synthesis: CTP was synthesized with solid-phase peptide synthesis (SPPS) method and characterized by reverse-phase high performance liquid chromatography (HPLC) and MALDI-TOF Mass spectroscopy. 2) Synthesis of CTP-conjugated Targeting Polymer: PLGA-PEG-MAL copolymer was conjugated to CTP through a thiol–maleimide reaction. The formation of polymer – peptide conjugate was confirmed by H-NMR spectroscopy. 3) Synthesis and characterization of CTP targeted polymeric NPs: CTP NPs were synthesized by single- step nanoprecipitation. Synthesized NPs were characterized in size via Dynamic Light Scattering (DLS). 4) Cellular uptake studies in neonatal rat cardiomyocytes: Neonatal rat cardiomyocytes were cultivated in fluorescence compatible plate and treated with CTP NPs. Internalization CTP NPs by cardiomyocytes was visualized by confocal laser scanning microscopy.
Conclusion: Designing cardiac-targeted NPs may provide new treatment opportunities for various cardiac conditions such as cardiac arrhythmias and heart failure.

Acknowledgments: Montreal Heart Institute

Odd Posters

Presenter: Vicky Wang 09h45/ PhD Jr./P21

Title: Effects of organophosphate esters on a human granulosa cell line

Authors: Wang, X. (Vicky); Robaire, B.; Hales, B. F.

Abstract: Use of the polybrominated diphenyl ether (PBDE) flame retardants has been regulated due to their adverse health effects. The phasing out of PBDEs has resulted in their replacement by organophosphate esters (OPEs) but little information is available on their safety. A recent study found that maternal exposure to OPEs was associated with poor pregnancy outcomes, suggesting that OPEs may be detrimental to female fertility. Here we focused on the effects of commonly used OPEs on ovarian granulosa cells. To test the hypothesis that OPEs alter the cellular characteristics of granulosa cells to a lesser extent than PBDEs, we assessed the effects of three commonly used OPEs in comparison to those of a major PBDE, 2,2',4,4' tetrabromodiphenyl ether (BDE-47). The three OPEs we studied are: tris(methylphenyl) phosphate (TMPP), triphenyl phosphate (TPHP) and isopropylated triphenyl phosphate (IPPP). KGN cells, immortalized human granulosa cells, were exposed to an OPE or BDE-47 at a range of concentrations (0.001 – 100 μM) for 48h. Effects on cell counts, lysosome numbers, and lipid droplets were determined using high content imaging and fluorescent dye combinations. Treatment with BDE-47 and all three OPEs caused concentration-dependent decreases in cell survival, with IC50 values of 147 μM (BDE-47), 47.4 μM (TMPP), 155.5 μM (TPHP), or 39.7 μM (IPPP). The numbers of lysosomes decreased after exposure to BDE-47 (IC50= 45.1 μM), TMPP (IC50= 9.4 μM), or IPPP (IC50= 83.5 μM) for 48h; TPHP had no effect. The numbers of lipid droplets increased significantly after exposure to low concentrations of TMPP (5 μM), IPPP (3.2 μM) or TPHP (10 μM), whereas BDE-47 exhibited effects only at 100 μM. Thus, some of the OPEs we tested display toxicity endpoints in KGN cells at lower concentrations than BDE-47, suggesting that these alternative chemicals may be more toxic than those that they are replacing.

Acknowledgments: Supported by funding from CIHR; McGill University. We should like to thank Dr. Nicolas Audet for his technical support.

Odd Posters

Presenter: Kyla Bourque 09h45/ PhD Sr./P23

Title: Modeling GPCR conformation and function in iPSCs and their differentiated derivatives

Authors: Bourque, Kyla; Devost, Dominic; Pétrin, Darlaine; Hébert, Terry

---

Abstract: Numerous biosensor-based in-cell assays aim to better characterize signaling downstream of GPCRs as this superfamily represents the most druggable class of membrane receptors. Shifts in the availability of ions, partner proteins, lipids and other biomolecules in the local environment of a GPCR can alter biological activity. Accordingly, for cell-based assays to be translationally relevant with practical application, the biosensor must be situated in and mimic cell context with disease relevance. Our objective is to explore how cell context influences GPCR activity by monitoring receptor conformation upon agonist stimulation. With improvements in fluorescent probe chemistry, the conjugation of receptors with fluorescent reporter proteins allows for the generation of biosensors that can monitor receptor activation and function. To build a biosensor line that reports on conformational changes, we introduced fluorescent biarsenical hairpin binder sites (FlAsH) as acceptors for bioluminescence resonance energy transfer. We inserted multiple FlAsH tags along the intracellular surface of the AT1R and β2AR along with a C-terminally fused Renilla luciferase to monitor the conformation of the receptor upon ligand binding. To investigate how cell context influences a GPCRs activity in intact live cells, we will use inducible pluripotent stem cells as a model due to their capacity to differentiate into any cell lineage. Our conformation-sensitive biosensors will be introduced into the AAVS1 safe harbour site and then differentiated into cardiomyocytes, vascular smooth muscle cells and neurons. To date, we have successfully generated beating cardiomyocytes that express relevant cell-specific markers troponin T and α-actinin. Once validated, our system using receptor conformation as a readout can guide rational drug discovery as we believe our sensors will be able to discriminate different types of agonists by potency and efficacy, proving to be useful tools for drug discovery.

Acknowledgments: This project is funded by the Canadian Institutes of Health Research (CIHR).

Odd Posters

Presenter: Colin Cheng 09h45/PhD Sr./P25

Title: IL-1 receptor modulators protect against preterm birth and retinopathy of prematurity

Authors: Cheng, Colin; Geranurimi, Azade; Quiniou, Christiane; Xin, Hou; Tang, Zhu; Lubell, William; Chemtob, Sylvain

---

Abstract: The cytokine interleukin-1β (IL-1β) binds to the IL-1 receptor (IL-1R) and plays an integral role in inflammation. Notably, its signaling leads to parturition in preterm labour and contributes to retinal vaso-obliteration characteristic of retinopathy of prematurity. Therapeutics targeting IL-1β and IL-1R are approved to treat rheumatoid arthritis; however, all are large proteins with clinical limitations including immunosuppression and immunogenicity. Immunosuppression by such therapeutics is due in part to the suppression of NF-kB signalling, which is required for immuno-vigilance and cytoprotection.
The all-D-amino acid peptide 101.10 (RYTVELA) is an allosteric modulator of IL-1R which exhibits functional selectivity and conserves NF-kB signalling, yet inhibits other IL-1-activated pathways. It is effective in experimental models of preterm labour and oxygen-induced retinopathy. To improve our understanding of structure-activity relationships of 101.10, a panel of 12 derivatives was synthesized employing the stereoisomers of α-amino-γ-lactam (Agl) and α-amino-β-hydroxy-γ-lactam (Hgl) residues to constrain Thr-3 and Val-4. The influences of lactam mimic structure and configuration were examined in vivo in murine models of preterm labour and retinopathy of prematurity and in vitro in cell lines. Notably, all analogs did not inhibit NF-kB signalling. The efficacy of the derivatives in preventing preterm labour varied significantly along with their capacity to prevent retinal vaso-obliteration. This demonstrates that the stereochemistry of the lactam structures influence efficacy and modulation of IL-1 signalling is beneficial for curbing inflammation in preterm labour and retinopathy of prematurity. Our biased ligands can serve as selective probes for studying IL-1 signalling in disease. They are also promising leads in developing immunomodulatory therapies with improved ease of administration and preservation of the beneficial effects of NF-kB signalling.

Acknowledgments: McGill University for the Graduate Research and Travel (GREAT) Award that allowed me to present sections of this work at the Association of Research in Vision and Ophthalmology (ARVO) Annual Meeting 2018 in Honolulu.

Presenter: Jmaeff, Sean 09h45/ PhD Sr./P27

Title: Discovery and Characterization of GFRa1-independent RET agonists

Authors: Jmaeff, Sean; Sidorova, Yulia; Lippiatt, Hayley; Barcelona, Pablo; Nedev, Hinyu; Hancoco, Mark; Saarma. Mart; Saragovi, Uri

---

Abstract: Glial cell line-derived neurotrophic factor (GDNF) has been extensively researched as a potential therapeutic agent for a number of degenerative diseases, including Parkinson's, ALS, and eye conditions such as glaucoma and retinitis pigmentosa (RP). It functions via a multicomponent receptor system involving the GFRa1 and the RET tyrosine kinase to generate pro-survival signals through the Akt/Erk pathways. Indeed, GDNF has made its way to clinical trials but this has been met with discouraging results, as with many large proteins used therapeutically. We hypothesize that the failures of GDNF stem from two major drawbacks: One being poor pharmacokinetics, and the other being the requirement for the GFRa1 co-receptor, thereby limiting the target cell population. To address this, we have screened and characterized a novel family of small molecules that activate RET directly, independent of GFRa1 expression. These compounds promote RET-dependent survival in cell lines and in degenerating retinas ex vivo. This work adds to the body of knowledge surrounding RET signaling, and validates it as direct therapeutic target with the possibility to improve degenerative phenotypes.

Acknowledgments: This work was supported by a CIHR grant to HUS and the Lundbeck Foundation and Sigrid Jusélius Foundation to MS. We are thankful for reagents provided by Dr. Brian Pierchala (pRET antibodies), Dr. Eero Castren (MG87/Trk cell lines), Dr. Carlos Ibanez (MG87/RET cell lines), and Dr. T. Li (RHOP347S transgenic mice). Jenni Montonen assisted with the Ret phosphorylation assays. Dr. Enrique de la Rosa assisted with retinal explants and TUNEL assays. Ms. Lucia M. Saragovi counted and quantified TUNEL assays.
The McGill SPR-MS Facility thanks the Canada Foundation for Innovation (CFI grant #228340) for the BIACORE T200 SPR infrastructure.

Presenter: Gauthier Schang 09h45/ PhD Sr./P29

Title: Loss of GATA2 impairs FSH production in male mice in vivo

Authors: Schang, Gauthier; Brûlé, Emilie; Boehm, Ulrich; Bernard, Daniel J.

Abstract: Mammalian reproduction is dependent on follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secreted by pituitary gonadotrope cells. The dimeric hormones share a common α-subunit (CGA) linked to hormone-specific β-subunits (LHβ and FSHβ). Relative to LHβ, the mechanisms regulating FSHβ (Fshb) synthesis are poorly resolved. Activins are selective regulators of Fshb transcription and act via the transcription factors FOXL2, SMAD3, and SMAD4. However, these proteins are co-expressed in other tissues that do not produce FSH. As such, there must be some other yet unidentified factor(s) that endow gonadotropes with the unique ability to express Fshb. We were particularly interested in GATA2, as conditional deletion of Gata2 in mouse pituitaries was previously shown to decrease serum FSH levels in male mice. We hypothesized that GATA2 plays a necessary role in Fshb expression in gonadotropes. We generated gonadotrope-specific GATA2 knockouts by crossing floxed Gata2 animals to mice in which Cre recombinase is expressed from the endogenous GnRH receptor locus, leading to specific genetic recombination in gonadotropes. These animals produced ~50% less FSH compared to littermate controls. In contrast, females had normal fertility, cyclicity, and FSH production. This sex difference suggested a potential role for sex steroids. However, castrated cKO males still produced less FSH compared to castrated controls, indicating that androgens did not account for the sex-specific phenotype. Ultimately, these data show that GATA2 plays an important role in quantitatively normal FSH production in males, but not in females.

Acknowledgments: The authors would like to thank undergraduate students Nittha Lalang, Jonas Lehnert, and Erica Wu for their assistance, and Ying Wang (McGill University, Montreal, Canada) for her technical assistance with the Milliplex assay. This research was funded by: CIHR MOP-133394 to DJB; CIHR fellowship GSD 152308, FRQS fellowship 31338, and Dr. Samuel Solomon Fellowship in Endocrinology to GS; and NSERC CGSM fellowship to EB

Presenter: Issan Zhang 09h45/PhD Sr./P31

Title: Sahaquine: a novel hybrid molecule for glioblastoma multiforme

Authors: Zhang, Issan; Beus, Maja; Stochaj, Ursula; Zorc, Branka; Maysinger, Dusica

---

Abstract: Glioblastoma multiforme (GBM) is the most common and deadly of brain tumors. Current chemotherapy with temozolomide shows considerable limitations in primary GBM and is largely ineffective in recurrent tumors. Histone deacetylase 6 (HDAC6) has emerged as a valuable target in cancer, as it promotes tumorigenesis, tumor survival and invasiveness. Pan-HDAC inhibitors (e.g. vorinostat) have been investigated in clinical trials for GBM but caused serious side effects due to a lack of selectivity. We present here the mechanisms of action of sahaquine, a novel hybrid molecule that selectively inhibits histone deacetylase 6 (HDAC6), which is highly upregulated in GBM compared to normal brain tissues. **Hypothesis:** 1) Sahaquine is superior to temozolomide in killing GBM tumoroids and brain tumor stem cells. 2) Sahaquine-mediated HDAC6 inhibition impairs cytoskeletal organization, motility and signaling of key survival pathways (AKT, ERK1). **Results:** Sahaquine treatment caused the selective hyperacetylation of HDAC6 substrates (i.e. α-tubulin), thereby impairing cytoskeletal organization and cell motility. The primaquine moiety of sahaquine reduced P-glycoprotein activity, improving intracellular drug retention. Sahaquine is more effective than temozolomide in killing GBM in 2D and 3D models (tumoroids and brain tumor stem cell aggregates), and inhibiting markers of GBM aggressiveness (EGFR levels and activation of AKT, ERK1). **Conclusion:** Sahaquine-induced GBM cell death is significantly increased in combination treatments with TMZ, buthionine sulfoximine and quercetin. These combinations merit further investigations in patient-derived organoids, and eventually in humans.

Acknowledgments: The authors wish to thank NSERC and CIHR for their financial support.

Odd Posters

POSTER SESSION II
14h00 – 15h30

P2	Amanda Perozzo	MSc Junior	IPN
P4	Yuhao Yan	MSc Junior, other department	IPN
P6	Valerie Bourassa	PhD Senior	IPN
P8	Shafqat Rasool	PhD Senior	BIOCHEM
P10	Ryan Alexander	PhD Senior	IPN
P12	Haley Deamond	MSc Junior	Pharmacology
P14	Sasen Efrem	MSc Junior	Pharmacology
P16	Xixiao Li	MSc Senior	Pharmacology
P18	Mariana Asslan	PhD Junior	Pharmacology
P20	Heather Fice	PhD Junior	Pharmacology
P22	Sarah Mackinnon	PhD Junior	Pharmacology
P24	Courtney Smith	PhD Junior	Pharmacology
P26	Jennifer Chen	PhD Senior	Pharmacology
P28	Morgan Foret	PhD Senior	Pharmacology
P30	Jace Jones-Tabah	PhD Senior	Pharmacology
P32	Han (Aileen)Yan	PhD Senior	Pharmacology
P34	Luisina Ongaro Gambino	Postdoctoral Fellow	Pharmacology

Presenter: Amanda Perozzo 14h00/MSc Jr-IPN/P2

Title: Auxiliary proteins target distinct regions on AMPARs to modulate receptor function

Authors: Perozzo, Amanda; Arsenault, Marika; Aurousseau, Mark; Bowie, Derek

---

Abstract: AMPA receptors (AMPARs), a family of ligand gated ion channels, are fundamental for synaptic transmission across all brain regions. Recent work has identified that the AMPAR pore forming subunits co-assemble with a variety of auxiliary proteins. Proteomic analysis suggests that AMPAR signaling complexes are made up of at least 3 families of transmembrane proteins, namely the claudin family of proteins including TARPs and GSG1L, the cornichon homologs (CNIHs) and the CKAMP family. These accessory proteins are garnering much interest as they have been shown to not only regulate the trafficking of receptors into and out of the synapse, but also directly affect their functional behaviour. However, the underlying structural basis for auxiliary protein modulation of AMPARs is poorly characterized. We have identified a hotspot on the AMPAR ligand-binding domain (LBD) that governs auxiliary protein interactions with AMPARs. Our data show that the electropositive KGK motif, a conserved extracellular domain previously identified by our lab, binds exclusively to Type I and Type II TARPs, as well as GSG1L. In contrast, an electronegative region on AMPARs is responsible for CNIH-3 modulation of the receptor. Furthermore, we show that by binding to distinct regions on the receptor, TARPs and CNIHs are able to modulate AMPARs through different gating mechanisms. CKAMP44 is unaffected by mutation of either of these regions, suggesting that this protein exerts its effects via other sites on the channel. In summary, this work establishes that auxiliary proteins modulate AMPARs by targeting distinct structural binding sites, which may be significant for drug development and therapeutics.

Acknowledgments: CIHR, NSERC

Presenter: Yuhao Yan 14h00/MSc Jr-IPN/P4

Title: GluA2-Containing AMPA Receptors Rendered Calcium-Permeable by Auxiliary Proteins

Authors: Yan, Yuhao; Osswald, Ingrid; Brown, Patricia; Bowie, Derek

---

Abstract: Ca2+ influx through ionotropic glutamate receptors (iGluRs) in synapses is crucial in mediating fundamental cellular processes found in neurons such as long-term potentiation (LTP). It has been generally assumed that synaptic Ca2+ influx is dominantly mediated through NMDA receptors (NMDAR), largely because of their high permeability to Ca2+ and the low abundance of calcium-permeable AMPA receptors (CP-AMPAR) in most synapses. Here we show that AMPARs can also contribute to Ca2+ influx independent of NMDAR in certain cell types in mouse retina, cerebellum and hippocampus. Surprisingly, a subpopulation of these CP-AMPARs are philanthotoxin and IEM insensitive. Such pharmacological profile suggests that this subpopulation consists mainly of GluA2-containing AMPARs, which goes against previous assumptions that GluA2-containing AMPARs are calcium impermeable. In heterologous expression system, we show that GluA1/A2 heteromers alone do not permeate calcium; however, the presence of auxiliary proteins, namely TARP-□2 and Cornichon-3 (CNIH3), render them calcium-permeable. This finding provides yet another molecular mechanism of how synaptic Ca2+ influx as well as downstream cellular pathways can be regulated.

Acknowledgments: This work is funded by FRQS master award and CIHR grant.

Presenter: **Valerie Bourassa** 14h00/PhD Sr-IPN/P6

Title: Internalization of the substance P receptor following treadmill exercise in an ankle joint model of osteoarthritis

Authors: Bourassa, Valerie; Deamond, Haley; Ribeiro-da-Silva, Alfredo

---

Abstract: The internalization of the receptor for substance P, the neurokinin-1 receptor (NK-1r) on lamina I projection neurons of the spinal dorsal horn has been used as a marker of nociceptive responses. Lamina I is an important centre for the modulation and forwarding to the brain of pain-related information. Therefore, changes in the properties of lamina I projection neurons may be important for pain. Previous work from our lab has shown de novo expression of NK-1r on lamina I pyramidal neurons in both neuropathic pain and inflammatory arthritis models. In addition to this, we have shown that forced movement of an inflamed joint or intradermal injection of capsaicin leads to NK-1r internalization. This project aims to investigate whether internalization of the receptor on NK-1r positive neurons will occur in a rat ankle joint model of osteoarthritis (OA) after a treadmill exercise. Using rats of both sexes, OA was induced using an injection of the glycolytic inhibitor mono-iodoacetate (MIA) into the right tibio-talar joint. At 8 weeks post MIA injection, a time where OA-related pain is consistent and irreversible, animals were forced to run for 10 minutes at a speed of 15 m/min on a treadmill and immediately perfused. NK-1r internalization was then analyzed using immunocytochemistry and confocal microscopy. Qualitative analysis of our results show that running MIA animals underwent significant internalization of the NK-1r as compared to non-running MIA animals and vehicle-injected controls. This indicates that movement during a treadmill exercise results in pain, which is of significance as pain during movement is a major complaint of osteoarthritis patients.

Acknowledgments: Valerie Bourassa is a recipient of a Louise Edwards Foundation Doctoral Studentship. Haley Deamond is a recipient of studentship from the McGill University Faculty of Medicine. This work was funded by Canadian Institutes of Health Research Operating Grant MOP-136903.

Presenter: Shafqat Rasool 14h00/PhD Sr-BIOC/P8

Title: Structural basis of PINK1 activation and ubiquitin phosphorylation

Authors: Rasool, Shafqat; Truong, Luc; Soya, Naoto; Lukacs, Gergely; Trempe, Jean-Francois

---

Abstract: Mutations in PINK1 cause autosomal recessive Parkinson's disease (PD), a neurodegenerative movement disorder. PINK1 is a kinase that acts as a sensor of mitochondrial damage and initiates Parkin-mediated clearance of the damaged organelle. PINK1 phosphorylates Ser65 in both ubiquitin and the ubiquitin-like (Ubl) domain of Parkin, which stimulates its E3 ligase activity. This year we published a study in which we established that PINK1 autophosphorylation is necessary for substrate recognition. This autophosphorylation takes place at a conserved Ser205 in Tribolium Castaneum PINK1 (Ser228 in human PINK1). We also demonstrate that human PINK1 autophosphorylation at Ser228 is necessary for the generation of phospho-ubiquitin chains under conditions of mitochondrial damage in human cells to trigger Parkin activation. Analysis of the recently published crystal structures in light of our hydrogen-deuterium exchange mass spectrometry data allowed us to create a thermodynamic model for PINK1 activation via autophosphorylation to yield a conformation that is competent for ubiquitin binding. Our recent phosphorylation experiments also provide insights into the kinetics and structural determinants of autophosphorylation. Currently, we are employing different approaches to obtain the crystal structure of the PINK1 autosphosphorylation complex.

Acknowledgments: Nathalie Croteau, Michel Therien, Gian-Luca McLelland

Even Posters

Presenter: Ryan Alexander 14h00/ PhD Sr-IPN/P10

Title: Cerebellar stellate cell excitability is regulated by coordinated shifts in voltage-gated Na+ and K+ channels

Authors: Alexander, Ryan; Mitry, John; Sareen, Vasu; Khadra, Anmar; Bowie, Derek

---

Abstract: Although it is generally assumed that basic neurophysiological properties measured in vitro using whole-cell patch-clamp electrophysiology are comparable to their behaviour in vivo, there have been few attempts to characterize changes induced during patch-clamp investigation. Using whole-cell patch clamp in acute cerebellar slices, we observed drastic increases in excitability in both cerebellar stellate cells and granule cells, but not in Purkinje cells, over a 25 minute recording duration. In stellate cells, this phenomenon was accompanied by a decrease in spike latency and hyperpolarization of action potential (AP) threshold. Using an augmented Hodgkin-Huxley firing model, we predicted modulation of both voltage-gated sodium channel (Nav) and potassium channel (Kv) gating properties, with a primary role for the shift of Nav. Measuring these isolated responses in whole-cell voltage-clamp configuration confirmed that Nav voltage-dependence of activation as well as channel availability shift substantially over the course of the experiment, whereas delayed rectifier Kv gating remained stable. Surprisingly, large shifts were also observed in A-type Kv properties that affect AP frequency but not AP threshold. These findings demonstrate the challenges associated with this experimental technique, and the susceptibility of certain cell types for dramatic changes in basal functional properties. Furthermore, they also suggest new modes of long-term plasticity of excitability mediated through modulation of Nav channels.

Acknowledgments: CIHR, NSERC, Savoy Foundation, McGill Faculty of Medicine

Presenter: Haley Deamond 14h00/ MSc Jr./P12

Title: The Role of the Immune System in the Sexual Dimorphism's of Osteoarthritic Pain

Authors: Haley Deamond, Valerie Bourassa, Alfredo Ribeiro-da-Silva

---

Abstract: Arthritis is a disease that impacts 4.6 million Canadians costing the Canadian health care system $33 billion annually. The most common form of Arthritis is Osteoarthritis (OA) which causes pain, tenderness, stiffness, inflexibility, and bone spurs. Clinically, there is an increased risk, prevalence, incidence and severity of this disease in women. Indeed, 2/3 of the diagnoses are in women. In addition to this, females with similar levels of joint degradation as males report higher pain. Other sexual dimorphisms have been outlined in the pain literature where different immune cells mediate pain in males and females. It has been shown, at the level of the spinal cord in mice, that mechanical allodynia is preferentially mediated by microglia in males in contrast to T-cells in females, when using a neuropathic pain model. Despite this evidence, little research has been done to investigate the roles of immune cells in the periphery in OA. Thus, this project aims to examine pain severity and onset in both male and female rats, the level of degradation in both sexes, and the differential the role of macrophages, a known inflammatory mediator, in the maintenance of pain. Behavioural assessments showed no difference between males and females in pain severity and onset; however, female joints showed a trend towards greater levels of degradation of cartilage compared to males. Immunohistochemistry demonstrated that macrophages do not play a role in maintaining pain in OA at a late time point, although the possibility remains that they do at an earlier stage. In conclusion, this project confirms that more research needs to be done to investigate the sexual dimorphism of pain and more specifically the key immunological players in Osteoarthritic pain.

Acknowledgments: Haley Deamond is a recipient of studentship from the McGill University Faculty of Medicine. Valerie Bourassa is a recipient of a Louise Edwards Foundation Doctoral Studentship. This work was funded by Canadian Institutes of Health Research Operating Grant MOP-136903.

Even Posters

Presenter: Sasen Efrem 14h00/ MSc Jr./P14

Title: The 'Cholesteryl ester transfer protein' in Alzheimer's disease models

Authors: Efrem, Sasen; Munter, Lisa

Abstract: Background: As AD has strong associations with cholesterol metabolism and the brain, the most cholesterol-rich organ in the body, it is imperative to study the disease using animal models with analogous lipid metabolism to that of humans with AD pathology. One of the most overlooked elements of human lipoprotein metabolism is the cholesteryl ester transfer protein (CETP), a protein that is not expressed in wild-type mice. CETP is a regulator of HDL (high density lipoprotein) and LDL (low density lipoprotein) levels in humans: it transfers cholesterol esters from HDL to LDL particles and thus increases the amount of LDL-C in the plasma.
Hypotheses: Aβ production is enhanced in the presence of CETP and this effect can be reduced with CETP inhibitors or using CETP with loss-of-function mutations. Furthermore, this CETP-mediated increase in Aβ production will be greater in the presence of ApoE4, relative to ApoE3.
Methods: We hope to elucidate the mechanisms of CETP-mediated Aβ deposition using in vitro mouse astrocyte/sy5y co-culture assays and to associate CETP to the various ApoE isoforms.
Results: Human ApoE3 and ApoE4-expressing mouse astrocytes have been successfully stably transfected with a CETP plasmid; co-cultures using these astrocytes and SH-SY5Y cells over-expressing APP show increases in Aβ40 production compared to wild-type, however, the degree of increase does not appear to be isoform-specific. Further co-culture experiments will be done using ApoE3 and ApoE4-expressing astrocytes stably or transiently expressing CETP with APP over-expressing SH-SY5Y cells under different conditions (CETP inhibitor, LDL-receptor blocker); levels of secreted Aβ40 peptides will then be quantified by ELISA and MSD for Aβ42.

Acknowledgments: Dr. Münter, Felix Oestereich, Sandra Paschkowsky, Sherilyn Recinto, Jackie Hsiao Jing Lian, Committee Members: Dr. Bernard, Dr. Trempe, Dr. Hamel, Collaborators: Dr. Claudio Cuello, Dr. Judes Poirier. Special thanks to Dr. David Holtzman (Washington University, St. Louis) for ApoE-expressing mouse astrocytes and to Michal Zielinski for help with the AKTA

Presenter: Xixiao Li 14h00/MSc Sr./P16

Title: Mechanical stress induced differential gene expression responses in atrial and ventricular fibroblasts

Authors: Li, X.; Garcia-Elias, A.; Qi, X; Xiao, J; Nattel, S.

Abstract: Atrial fibrillation, the most common cardiac arrhythmias, occurs with the increase of atrial blood flow and atrial pressure, meanwhile leading to hemodynamic overload and excessive stretch.
Some connections have been found between pressure overload and the pathogenesis of cardiac remodeling. Studies show that atrial fibroblast, compared with ventricular fibroblasts, are more reactive and sensitive in response to various stimuli. For example, in a variety of models of congestive heart failure, an extensive atrial fibrotic response occurs with comparatively little ventricular fibrosis. Nevertheless, the differences in stretch-triggered genetic expression responses between atrial and ventricular fibroblasts and the molecular mechanisms underlying the development of atrial fibrillation have not been well described.
To investigate the differences between the stretch-triggered genetic expression of atrial and ventricular fibroblasts, we want to develop an in vitro cardiac fibroblasts stretch model to mimic pressure overload which causes fibrotic responses.
Cyclic uniaxial stretch was applied to rat cardiac fibroblasts cultured on silicone chambers and possible factors were tested, including coating matrix, FBS concentration of the medium, stretch elongation and stretch frequency. One reproducible experimental condition was found under which the mRNA level of collagen3a1 and alpha-smooth muscle actin (alpha-SMA) showed significant increases with stretch stimuli. Other fibrosis-related genes also responded to the mechanical stress (TGF-beta-2 and lysyl oxidase (LOX) decreased and LOXL1 and LOXL2 increased significantly).
After the condition being settled, we further began to study the differences between the stretch-triggered genetic expression of atrial and ventricular fibroblasts with RT-qPCR, immunoblotting and other related approaches.

Acknowledgments: This work is supported by Montreal Heart Institute.

Even Posters

Presenter: Mariana Asslan 14h00/ PhD Jr./P18

Title: Tumorigenic Roles of TLR Signaling Network in Lymphoid Neoplasms

Authors: Rousseau, Simon

Abstract: Lymphoid neoplasms are a family of cancers affecting B-cells, T-cells, and NK cells. In lymphoid neoplasms, the Toll-Like Receptor (TLR) signaling adapter molecule MYD88 is the most frequently mutated gene. This signaling adaptor relays signals from TLRs to transforming growth factor-β–activated kinase1 (TAK1). TAK1 is a crucial regulator of cell survival pathways such as NFκB and MAPK pathways. Therefore, investigating the TLR-signaling pathway in lymphoid neoplasms is critical to understand the pathogenesis of lymphomas and identify new therapeutic targets to treat them. In this study, we hypothesize that blocking signaling downstream of MYD88 will impair lymphoid neoplasms survival. Therefore, we will target TAK1, IKK, TPL2, MKK1/MKK2, JNK, and p38 either by genetic downmodulation (using CRISPR-Cas9 system) or pharmacologic inhibition in Waldenstrom's Macroglobulinemia (WM) lymphoma cells injected in a zebrafish model. In WM, >95% of cells harbor gain-of-function mutation in MYD88, making this lymphoma an excellent model to study TLR-mediated cellular transformation. Preliminary experiments have shown that pharmacological inhibition of TAK1 by 5Z-7-oxozeaenol at 1 μM can decrease WM cells proliferation by 53% in vitro, while the same dose is non-toxic for embryos up to 5 days. Lymphoma cells will be injected into zebrafish embryos at 2 days post fertilization. Afterwards, the embryos will be exposed to the pharmacological inhibitors via continuous waterborne exposure. Proliferation of lymphoma cells will be monitored by live confocal imaging of zebrafish embryos at different time points. Eventually the embryos will be dissociated to a single cell suspension and the number of cells will be counted. This study will provide further insights of the molecular mechanisms driving TLR-mediated tumor formation, and their potential application to treat patients with lymphoid neoplasms.

Acknowledgments: This project is supported by The Natural Sciences and Engineering Research Council of Canada

Presenter: **Heather Fice** 14h00/PhD Jr./P20

Title: Effects of Aging on Male Rat Germ Cell Telomere Dynamics

Authors: Fice, Heather; Robaire, Bernard.

Abstract: Delayed parenthood is a current trend in society. Although maternal age and offspring health have been well characterized, the paternal influence remains largely understudied. However, several studies have shown a correlation between increased paternal age and detrimental outcomes in the offspring, such as achondroplasia, bipolar disorder, and autism spectrum disorders. As many of the observed disorders involve multiple genes, it can be hypothesized that the source of these problems stems from a disruption of chromatin integrity. This disruption may come from a variety of sources including: oxidative stress, accumulating DNA damage, and altered telomere length. Studies on telomere length in sperm from aged males have been conducted in mice and humans, with conflicting results. We hypothesize that with advanced paternal age, the telomeres of rat germ cells decrease in length, causing an overall change in chromatin structure. Sprague-Dawley rats have been aged to 4 and 18 months, to obtain representative young and aged populations. Germ cells from various stages of spermatogenesis were collected, including sperm from the caput and cauda epididymidis. The absolute telomere length of sperm from the cauda epididymidis was found to be 299kb (SEM=44) for young and 231 kb (SEM=28) for aged rats. This decrease is consistent with the telomere length changes observed in mice. Fluorescence in situ hybridization for telomeres has been done on perfusion fixed testes, and will be quantified. To complement these experiments, telomerase activity will be measured by TRAPeze assay and western blot analysis. Taken together, these results suggest how telomere length, telomerase activity, and telomere organization may change with age. Understanding germ cell telomere dynamics is of critical importance when considering chromatin structure and embryo development given the genetic outcomes observed in the offspring of older men.

Acknowledgments: We would like to thank Dr. Aimee Katen for her assistance with the methods development. This research has been supported by the CIHR Institute for Gender and Health.

Presenter: **Sarah MacKinnon** 14h00/PhD Jr./P22

Title: Spt5 promotes efficient transcription termination to antagonize heterochromatin

Authors: MacKinnon, Sarah; Tanny, Jason

Abstract: A detailed characterization of the regulation of RNA Polymerase II (RNAPII) is necessary to understand diseases associated with aberrant transcription, including cancer, cardiac hypertrophy and HIV. Processivity of RNAPII is regulated by Spt5, an essential elongation factor that couples transcription to RNA processing and chromatin modifications. The Spt5 C-terminal domain (CTD) regulates transcription termination, with threonine-to-glutamate (T1E) mutations causing transcriptional read-through. The mechanisms through which the CTD couples transcription to other processes are not understood. To address this gap, we completed a genetic screen to identify mutants that enhance phenotypes of CTD mutants as a means to functionally characterize the CTD. Fission yeast was used as a model, as its epigenome and transcription regulation is similar to mammalian cells.

The screen uncovered genetic interactions between Spt5 CTD mutants and heterochromatin boundary proteins. Defective boundaries can lead to an overabundance of heterochromatin and aberrant gene silencing. Thus, I hypothesized that the CTD is required for heterochromatin boundary function, and predicted that the mutant lacking the CTD (Spt5ΔC) would exhibit increased heterochromatin.

Methylation of histone H3 lysine 9 (H3K9me) is a conserved marker of heterochromatin. Anti-H3K9me chromatin immunoprecipitation (ChIP) in Spt5ΔC cells established that heterochromatin had spread past the boundaries. Reporter gene assays indicated that increased H3K9me was indeed associated with decreased gene expression. Thus, the Spt5 CTD antagonizes heterochromatin spreading. Defective termination similar to that seen in Spt5-T1E cells is linked to increased heterochromatin. ChIP in mutants with defective termination exhibited increased H3K9me. We propose that the CTD recruits a termination factor to restrict heterochromatin. This project will provide insight into how Spt5 regulates heterochromatin and prevents gene silencing.

Acknowledgments: Funded by NSERC

Even Posters

Presenter: Courtney Smith 14h00/PhD Jr./P24

Title: Characterization of the IGSF1 Interactome

Authors: Smith, Courtney L; Bernard, Daniel J

Abstract: The hypothalamic-pituitary-thyroid (HPT) axis controls the synthesis and secretion of thyroid hormones (THs). THs have many actions throughout the body, but are best known for their regulation of growth and metabolism. Central hypothyroidism, a rare endocrine disorder, occurs when defects in the hypothalamus and/or the pituitary cause TH-deficiency. Mutations in the immunoglobulin superfamily, member 1 (IGSF1) gene are the most common cause of congenital central hypothyroidism. IGSF1 is a type 1 transmembrane protein of unknown function that is highly expressed in the pituitary gland. To define potential mechanism of IGSF1 action, we will identify its interacting partners using a new proximity interaction labelling method, BioID. The BioID method attaches a BirA* biotinylase onto the protein of interest (bait). In the presence of biotin, BirA* biotinylates interacting and proximal proteins. The biotinylated proteins (prey) are pulled down using streptavidin beads, which have a high affinity for biotin, and are identified using mass spectrometry. As we are most interested in intracellular signaling, we fused BirA* to the intracellular tail of IGSF1. The IGSF1-BirA* fusion protein properly traffics to the plasma membrane and the fusion protein possesses biotinylase activity. Next, we will stably express the IGSF1-BirA* fusion protein in homologous and heterologous cell lines and perform the proteomic screen. In the long-term, our work will define the function of IGSF1 in the pituitary, and may facilitate the discovery of novel causes of congenital central hypothyroidism.

Acknowledgments: Supported by CIHR, RQR, and McGill Faculty of Medicine.

Even Posters

Presenter: Jennifer Chen 14h00/ PhD Sr./P26

Title: Novel Interactions between H2Bub1 Regulators, Rtf1 and the PAF Complex, can be Modulated by DNA Binding

Authors: Chen, Jennifer; Mbogning, Jean; Page, Viviane; Tanny, Jason

---

Abstract: Histone modifications mark and maintain multiple chromatin states, with certain marks, such as histone H2B monoubiquitination (H2Bub1), exclusively associated with RNA polymerase II (Pol II) transcription. Impaired regulation of H2Bub1 is associated with various cancers, but the molecular mechanisms that couple H2Bub1 to transcription and its role in regulating gene expression are poorly understood. H2Bub1 is catalyzed by the E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase, but transcriptional deposition of H2Bub1 also requires the Polymerase-Associated Factor complex (PAFc) and Rtf1, both conserved regulators of transcription elongation and mRNA processing. To elucidate the mechanisms PAFc and Rtf1 use to couple histone modification to the Pol II elongation complex, I developed an in vitro system to study the protein-protein interactions connecting PAFc, Rtf1, and histone-modifying enzyme complexes using the model eukaryote fission yeast. Whereas previous studies showed interactions between these factors involving the N- and C-terminus of Rtf1, I focused on novel interactions involving the highly conserved Plus 3 domain. The Plus 3 domain is involved in Rtf1's recruitment to chromatin through an interaction with phosphorylated Spt5, a transcription factor involved in Pol II pause release and elongation. I have found novel interactors with the Plus 3 domain including specific subunits of PAFc and the E2/E3 complexes. The Plus 3 domain was also previously described to bind nucleic acids. Further analysis of the novel PAFc interaction indicates that it is mutually exclusive with this nucleic acid interaction, shedding light on a potential function for this binding. Additionally, we have identified mutations that perturb these interactions in vitro and are in the process of assessing effects in vivo. Our results highlight potentially novel functions and mechanisms of regulation for the Rtf1 Plus 3 domain in linking histone modification and Pol II transcription.

Acknowledgments: This work was funded by the Canadian Institute of Health Research (CIHR).

Even Posters

Presenter: Morgan Foret 14h00/ PhD Sr./P28

Title: Detection of Lipid Peroxidation in Primary Neuronal Cultures with a Novel Fluorogenic Probe

Authors: Foret, M.; Do Carmo, S.; Greene, L.; Lincoln, R.; Zhang, W.; Cosa, G.; Cuello, AC

Abstract: Reactive oxygen species (ROS) play an important role in health and disease. ROS are known to mediate cell signalling pathways but can also lead to pathological processes through oxidative damage. Studies have linked ROS to neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and ALS among others. Specifically, by-products of lipid peroxidation, a form of ROS, are elevated in the brains of individuals with neurodegenerative diseases.
Accurate quantification of ROS in vitro and in vivo proves to be difficult due to confounding factors associated with experimental conditions. Cell culture creates an oxidative environment and media contains factors that can induce oxidative stress or create artifacts that distort results or lead to false conclusions. However, the subtle and transient changes in ROS and lipid peroxidation levels are likely biologically significant and important for our understanding of pathological cascades.
To better understand the role of lipid peroxidation in neurodegeneration it is essential to establish reliable methods of quantifying levels in vitro and then in vivo. Towards this goal, we utilized a highly sensitive fluorogenic probe H4BPMHC to quantify lipid peroxidation in enriched primary neuronal cultures with different antioxidant exposures. Our goal was to validate this novel probe in primary neurons and our findings showed that neurons cultured without antioxidant in the days before exposure to stressor had increased lipid peroxidation levels when compared to those which had antioxidants the entire culturing period. These results highlight the importance of assessing cell culture conditions before conducting experiments that quantify oxidative stress. Furthermore, the results validate the capability of this probe to detect differences in lipid peroxidation levels in primary neurons and provide a basis for conducting experiments where lipid peroxidation changes in healthy and disease states can be investigated.

Acknowledgments: This work was supported by the Canadian Institute of Health Research.

Even Posters

Presenter: Jace Jones-Tabah 14h00/PhD Sr./P30

Title: Fiber-optic recording of FRET biosensors for detecting GPCR signalling in vivo

Authors: Jace Jones-Tabah, Paul B.S. Clarke, Terence E. Hébert

---

Abstract: G protein-coupled receptors (GPCRs) mediate neuronal responses to neurotransmitters and neuromodulators, and are major drug targets in neuropsychiatric disease. Individual GPCRs signal via multiple downstream effectors, only some of which may mediate therapeutic effects in vivo. Furthermore, the specific complement of signalling cascades engaged by a given GPCR is determined by several factors, including the particular ligand, as well as the cellular and tissue context. We have developed a method for recording Förster resonance energy transfer (FRET)-based biosensors that report signalling downstream of GPCRs in real time in live animals. We combine fiber-photometry-based fluorescent recording with a genetically-encoded FRET biosensor that reports protein kinase A activity with high spatial and temporal resolution. Biosensors are expressed in wild-type animals using viral vectors and cell-type selective expression is achieved using specific promoters. Flexible fiber-optic patch cords allow imaging to be performed in freely moving animals and will allow the simultaneous measurement of behavioral and signalling responses.

Acknowledgments: This work was supported by funding from CIHR, NSERC and McGill Healthy Brains for Healthy Lives.

Presenter: Han Yan 14h00/ PhD Sr./P32

Title: A comparison of the effects of two new generation flame retardants on markers of oxidative stress and cell cycle regulation during endochondral bone formation in the mouse limb bud culture model

Authors: Yan, H.; Hales, B.F.

Abstract: Flame retardants (FRs) are applied to many products to slow fire propagation. The use of “new generation” organophosphate esters (OPEs) as FRs has surged in the past 15 years, but knowledge regarding the potential consequences of exposure to these chemicals remains limited. Previously, we showed that several commonly used OPEs, including triphenyl phosphate (TPHP) and tert-butylphenyl diphenyl phosphate (BPDP), suppressed endochondral bone formation in the ex vivo mouse limb bud culture model. One of the effects observed was a reduction in the differentiation of proliferating chondrocytes into hypertrophic chondrocytes. The literature suggests that both a tightly controlled increase in reactive oxygen species (ROS) and an upregulation in the expression of cyclin-dependent kinase inhibitors (CKIs) play an important role in directing the exit of chondrocytes from the cell cycle and subsequently permitting their terminal differentiation. These changes must occur at the appropriate time and to the appropriate degree in order for proper bone formation to take place. We tested the hypothesis that OPE exposure alters the oxidative stress response and cell cycle regulation by assessing the expression of Hmox-1, an oxidative stress marker, as well as Cdkn1a and Cdkn1c, two CKIs, using qRT-PCR. Compared to control, 10 µM TPHP-treated limbs initially expressed lower levels of Hmox-1 and Cdkn1a followed by a sustained upregulation. Cdkn1c, on the other hand, was downregulated. In contrast, BPDP exposure did not affect Hmox-1 expression and had minimal effects on the two CKIs. Thus, although TPHP and BPDP are both detrimental to endochondral bone formation, they may have distinct mechanisms of action.

Acknowledgments: Supported by CIHR and FRQS.

Presenter: Luisina Ongaro Gambino 14h00/Postdoc./P34

Title: Regulation of human follicle-stimulating hormone β expression (FSHB) by activins, SMAD4, and FOXL2 in pituitaries of transgenic mice.

Authors: Ongaro Gambino, Luisina; Schang, Gauthier; Kumar, Rajendra; Treier, Mathias; Deng, Chu-Xia; and Bernard, Daniel J.

Abstract: Follicle-stimulating hormone (FSH) is an essential regulator of mammalian fertility. The hormone is synthesized by pituitary gonadotrope cells in response to GnRH and/or pituitary activins. Activins bind to activin type I/type II receptor complexes, which phosphorylate SMAD proteins. SMADs accumulate in the nucleus where they bind to FSHβ subunit (Fshb) promoter in combination with FOXL2. Female mice with gonadotrope-specific loss of Smad4 and Foxl2 are FSH deficient and sterile. It is presently unclear whether human FSHB expression is regulated by activins and, if so, by similar mechanisms. Human FSHB promoter-driven reporters are poorly responsive to activins in immortalized murine gonadotrope cells and SMAD/FOXL2 cis-regulatory elements identified in murine Fshb are not perfectly conserved in human FSHB. We used mice harboring a 10-kb human FSHβ transgene (hereafter hFSHB), expressing the corresponding mRNA and protein in the pituitary gland. We cultured pituitaries from hFSHB mice and measured murine Fshb and human FSHB mRNA expression. As expected, murine Fshb was stimulated by exogenous activins. Basal Fshb mRNA levels were greatly reduced by follistatin-288 (activin antagonist) or SB431542 (activin type I receptor inhibitor), demonstrating an essential role for endogenous activin signaling in murine Fshb expression. Activins stimulated and the inhibitors attenuated human FSHB, however the effects were smaller when compared with murine Fshb expression. Finally, we assessed whether FOXL2 and SMAD4 regulate human FSHB expression by crossing hFSHB transgenic mice with animals carrying floxed alleles for Foxl2 and Smad4. Ablation of FOXL2 and SMAD4 strongly impaired basal and activin-stimulated of both murine Fshb and human FSHB expression. Collectively, the data indicate that the human FSHB gene is activin responsive, and that its expression is dependent on FOXL2 and SMAD4. This suggests that mechanisms of Fshb/FSHB regulation may be conserved between species.

Acknowledgments: Supported by CIHR MOP-133394 to DJB and Ferring Inc. PDF Scholarship to LO