Pharmacology Research Day

26th Edition
May 20, 2022

McIntyre Medical Building
3655 Promenade Sir William Osler, Montreal, H3G 1Y6

Palmer Amphitheatre, Room 522 and 6th Floor Lobby

McGill | Department of Pharmacology and Therapeutics

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Pharmacology Research Day Committee

Dr. Maureen McKeague	Coordinator
Tina Tremblay	GPC and administrative assistant for the committee
Jasmine Phénix	PhD student
Rebecca Cummer	PhD student
Reilly Pidgeon	PhD student
Mozhdeh Mehdizadeh	PhD student
Grace Mazarura	MSc student
Alyson Jiang	MSc student
Dr. Etienne Billard	Postdoctoral researcher

Foreword

Dear colleagues,

After a two-year hiatus, we are excited to finally welcome everyone to the 26th edition of Pharmacology Research Day. We are eager to hear about the tremendous progress made by students of the Department of Pharmacology and Therapeutics and its associated departments in the last two years.

We would also like to welcome our keynote speaker, Dr. Jacques P. Tremblay. We look forward to hearing about his ongoing research in the field of gene therapy through his talk entitled *Development of gene correcting therapy for rare diseases*.

In this edition of PRD, a new presentation format will be available to students: flash talks. These Three Minute Thesis-style presentations are intended to give students the opportunity to rapidly present their research projects in a concise, yet infomative format. We hope you will enjoy the talks and posters presented throughout the day and will take the opportunity to network with your peers and learn about exciting research topics in pharmacology.

We would like to extend special thanks to event sponsors for their generous contributions, supporting staff (Tina Tremblay, Chantal Grignon, Anna Cuccovia, Cathy Shang Kuan, and Nadee Buddhiwickrama) who worked behind the scenes to make this event possible, and members of the organizing committee for their dedication and hard work in planning this in-person event.

Sincerely,

Students of the Pharmacology Research Day Committee

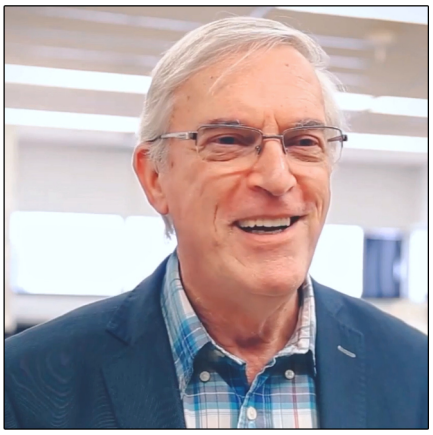
Back: Rebecca Cummer, Reilly Pidgeon, Etienne Billard
Front: Alyson Jiang, Grace Mazarura, Jasmine Phénix
Missing: Mozhdeh Mehdizadeh

Program

8h00-8h45	**Registration** - 6th Floor Lobby, McIntyre Med
8h45	**Pharmacology Research Day Introduction** - Palmer Amphitheatre, Room 522 *Dr. Maureen McKeague, PRD Chair*
9h00-10h00	**Keynote Address** - Palmer Amphitheatre, Room 522 *Professor Jacques P. Tremblay* Department of Molecular Medicine Faculty of Medicine, Université Laval Title: Development of gene correcting therapy for rare diseases
10h00-11h30	**Break & Poster Session I** - 6th Floor Lobby, McIntyre Med Odd Numbered Posters
11h30-13h00	**Oral Session I** - Palmer Amphitheatre, Room 522
11h30	Claire Lin, PhD - Bernard Lab
11h45	Mozhdeh Mehdizadeh, PhD - Nattel Lab
12h00	Joshua Emmerson, PhD – Cuello Lab
12h15	Jennifer Chen, PhD – Tanny Lab
12h30	Yanis Inglebert, Postdoctoral Fellow – McKinney Lab
13h00-14h00	**Lunch** - 6th Floor Cafeteria
14h00-15h30	**Poster Session II** - 6th Floor Lobby, McIntyre Med Even Numbered Posters
15h30-16h15	**Oral Session II** - Palmer Amphitheatre, Room 522
15h30	Adithi Sundarakrishan, PhD – Clarke Lab
15h45	Daniel Sapozhnikov, PhD – Szyf Lab
16h00	Etienne Billard, Postdoc – Hébert Lab
16h15-17h00	**3-Minute Flash Talk** - Palmer Amphitheatre, Room 522
	Andrew Bayne, PhD – Trempe Lab Evan Buddle, Incoming MSc – Bernard Lab Rebecca Cummer, PhD – Castagner Lab Bruktawit Maru, PhD – McKeague Lab Justin Meneses, PhD – Castagner Lab Jasmine Phénix, PhD – Münter Lab Reilly Pidgeon, PhD – Castagner Lab
17h00-18h00	**Awards Presentation/Closing Remarks** - 6th Floor Lobby, McIntyre Med

Keynote Speaker

Jacques P. Tremblay received a B.Sc. in Honors Biochemistry from McGill University in 1970, and a Ph.D. in Neuroscience from UCSD in 1974. From 1975 to 1976, he was a postdoctoral fellow at Laval university. Subsequently, he spent his entire career at Laval University where he is currently a full Professor of the Department of Molecular Medicine. He is currently a regular researcher at the Neuroscience Axis of the Research Center of CHUQ.



Jacques P. Tremblay, PhD

Full professor

Department of Molecular Medicine | Université Laval

His group has been working on cell and gene therapy treatment for Duchenne Muscular Dystrophy (DMD) since 1987. In 2006, Dr. Tremblay received the Henry Friesen Award from the Royal College of Physicians and Surgeons of Canada for his work on myoblast transplantation as a potential treatment for DMD.

Dr. Tremblay's group has also been conducting research on Friedreich's Ataxia since 2010. This disease is due to an elongation of the GAA trinucleotide repeat in intron 1 of the frataxin gene, which reduces expression of this protein. His group demonstrated that it is possible to remove the trinucleotide repetition by cutting with the CRISPR / Cas9 system before and after this repeat.

Tremblay's group also uses CRISPR / Cas9 technology to develop a treatment for Alzheimer's disease. This disease is due to the abnormal metabolism of the APP protein (Amyloid Precursor Protein) which leads to the formation of beta-amyloid peptides that form plaques. The formation of these peptides can be greatly reduced by the A673T mutation of the APP gene observed in a small portion of Iceland's population. Dr. Tremblay's group has demonstrated that this mutation could be produced with the CRISPR-derived Prime editing technology.

Sponsors

PRD Awards

Oral Presentation Award | Award for the best oral presentation ($250)

PhD Poster Award | Award for the best poster presentation by a PhD student in the Department of Pharmacology and Therapeutics ($250)

MSc Poster Award | Award for the best poster presentation by a MSc student in the Department of Pharmacology and Therapeutics ($250)

3-Minute Flash Talk Award | Audience choice award for the best 3-minute flash talk ($100)

Postdoc/RA/Tech Award | Award for the best presentation (oral or poster) by a postdoc/RA/Technician ($250)

Life Science Graduate Award | Award for the best poster presentation by a student supervised by a professor in the Department of Pharmacology and Therapeutics, but whose primary affiliation is not with Pharmacology ($100)

Judges

Dan Bernard	Faculty	Jason Tanny	Faculty
Derek Bowie	Faculty	Ajitha Thanabalasuriar	Faculty
Paul Clarke	Faculty	Jean-François Trempe	Faculty
Barbara Hales	Faculty	Bastien Castagner	Faculty
Terry Hébert	Faculty	Karima Alim	Postdoc
Jean-Sébastien Joyal	Faculty	Etienne Billard	Postdoc
Koren Mann	Faculty	Caroline David	Postdoc
Nathan Luedtke	Faculty	Martin Mackasey	Postdoc
Anne McKinney	Faculty	Ali Nejatie	Postdoc
Lisa Münter	Faculty	Simon Veyron	Postdoc
Bernard Robaire	Faculty	Sonia Do Carmo	Technical
John Gillard	Faculty	Luisina Ongaro	Technical

Oral Presentations

Format

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

Duration: 10 minutes.

Question period: 5 minutes.

Presentation format: Powerpoint. Recommended: 10 slides.

Schedule

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

11h30	Claire Lin, PhD - Bernard Lab
11h45	Mozhdeh Mehdizadeh, PhD - Nattel Lab
12h00	Joshua Emmerson, PhD – Cuello Lab
12h15	Jennifer Chen, PhD – Tanny Lab
12h30	Yanis Inglebert, Postdoctoral Fellow – McKinney Lab

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

15h30	Adithi Sundarakrishan, PhD – Clarke Lab
15h45	Daniel Sapozhnikov, PhD – Szyf Lab
16h00	Etienne Billard, Postdoc – Hébert Lab

Yeu-Farn (Claire) Lin

Loss of inhibin negative feedback in the pituitary leads to enhanced ovulation but pregnancy failure in mice

Yeu-Farn (Claire) Lin, Emilie Brûlé, Mitra Cowan, Loydie Jerome-Majeska, Ulrich Boehm, Herbert Y. Lin, Daniel Bernard

Follicle-stimulating hormone (FSH) is an essential regulator of mammalian reproduction, particularly in females. Inhibins are TGFβ family ligands made in the gonads that suppress FSH synthesis FSH by pituitary gonadotrope cells. Inhibins require a co-receptor, betaglycan or TGFBR3L, to mediate their functions. Female mice with a gonadotrope-specific deletion of betaglycan or global deletion of *Tgfbr3l* exhibit enhanced ovarian follicle development, numbers of ovulated eggs, and litter sizes compared to controls. Females with both co-receptors knocked out (hereafter dKO) show dramatic increases in FSH levels, ovary size, and eggs ovulated in natural cycles. dKO eggs are fertilization competent, the females become pregnant, and the number of implanted embryos at embryonic day 7.5 (E7.5) are significantly increased. Nevertheless, dKO females do not give birth to live offspring. By E10.5, the weight of the fetoplacental unit is decreased in dKO females, and many embryos display morphological abnormalities. By E14.5, most embryos in dKO females are dead and resorbing. Wild-type surrogates give birth to live young following transplantation of embryos from control or dKO females. Conversely, control but not dKO females carry wild-type embryos to term. These data suggest that the maternal environment in dKO mice cannot support successful pregnancies. Indeed, inhibiting estrogen production in pregnant dKO females with anastrozole increased the number of live embryos at E12.5, suggesting that estrogen is elevated during pregnancy and is detrimental to embryo development. FSH is also elevated during gestation. Both FSH and estrogen have been implicated in placental angiogenesis. We are currently investigating the morphology of the fetoplacental unit at E7.5 and E10.5 to determine whether aberrant placental development might contribute to infertility in dKO females. These experiments will show how loss of inhibin action from pituitary gonadotropes impedes embryo survival.

Aknowledgements | Funding sources: Supported by Canadian Institutes of Health Research and the Solomon Studentship Award (Division of Endocrinology, McGill University Health Centre)

The Role of Cellular Senescence in Atrial Fibrillation

Mozhdeh Mehdizadeh, Patrice Naud, Roddy Hiram, Feng Xiong, Issam H. Abu-Taha, Nhung Vuong, PhD,Eric Thorin, Gerardo Ferbeyre, Jean-Claude Tardif, Jean Francois Tanguay, Martin G.Sirois, Dobromir Dobrev, Stanley Nattel

Background: Age is a key determinant of the risk of atrial fibrillation (AF), but the underlying mechanisms are poorly understood. Cellular senescence is an aging and stress related response, however, its role in AF is unknown.

Objectives: 1) To evaluate AF susceptibility and the expression of senescence markers in the atrial tissue of rat models of aging and myocardial infarction (MI), a pathology that causes AF-promoting remodeling. 2) To study the effect of reducing the burden of senescent cells with senolytic therapy on AF susceptibility.

Methods: The AF-susceptibility of aged and MI rats was studied with transesophageal pacing, and optical mapping. The protein and gene expression of senescence markers and atrial fibrosis were evaluated. A combination of senolytics, dasatinib and quercetin (D+Q), was used to target senescent cells. Double staining of p16 and cardiac cell type markers was used to study which cardiac cell types are sensitive to D+Q therapy.

Results: Pacing-induced AF was seen in 100% of aged (20 months) vs 10% of young control rats (3 months), and in 87.5% of young rats subjected to MI. Conduction velocity was significantly slower in left atrium of aged (45±3 cm/s, $P=0.001$) and young MI rats (41±4 cm/s, $P<0.0001$) compared to young control rats (54±4 cm/s). Fibrosis was significantly greater in aged, and MI left atrium. An important senescence marker, p16, was upregulated in left atrium tissue of MI and aged rats. Intermittent senolytic therapy (D+Q) from the time of MI induction to 28 days later significantly reduced AF inducibility and decreased fibrosis in left atrium, while normalizing p16 expression in LA tissue, indicating that D+Q can reduce AF susceptibility.

Conclusion: Our study points to a significant role of cellular senescence in AF pathophysiology. Modulating cell senescence might provide the basis for novel therapeutic approaches in AF.

Aknowledgements | The authors thank Nathalie L'Heureux, Chantal St-Cyr, Yanfen Shi and Marie-Elaine Clavet for their technical support. Funding: CIHR,FRQS

Joshua Emmerson

Elucidating combined effects of amyloid and tau in aged McGill APPxR955-hTau rats: a novel bigenic model with Alzheimer's disease-like pathology

Joshua Emmerson, Sonia Do Carmo, Chunwei Huang, A. Claudio Cuello

Aims: There is an drastic need to better understand preclinical stages of Alzheimer's disease (AD) such as the pathological acceleration of amyloid and tau pathology in the brain. This study aimed at determining how and to which extent a combination of gradually developing amyloid and tau pathologies exacerbate each other in the context of Alzhiemer's disease-like pathology in a novel transgenic rat model named McGill-APPxR955-hTau.

Methods: Heterozygous McGill-R-Thy1-APP and R955-hTau transgenic rat models were crossed, producing a novel bigenic rat model with both amyloid and tau pathologies. Bigenic rats express human APP with the Swedish and Indiana mutations (KM670/671NL, V717F), as well as the longest isoform of human tau (2N4R) with the P301S mutation causative for frontotemporal dementia. Male and female transgenic rats were raised in cohorts to 12, 20 and 24 months of age and were subject to a battery of behavioural testing for cognition. Examination of amyloid and tau pathology was performed primarily using immunohistochemistry and Western blot. Bigenic rats were compared to single transgenics and wild-type littermate controls.

Results: APPxR955-hTau bigenic rats produced a gradual accumulation of human amyloid and tau, resulting in progressive cognitive deficits and modest pathology reflective of early stages. We discovered that the tau transgene produced a compensatory effect on cognition at 20 months, rescuing and dominating over amyloid-induced cognitive effects. However, at 24 months of age, bigenic rats displayed worsened cognition compared to single transgenics in spatial learning in memory. Reflective of these findings, bigenic rats developed exacerbated pathology at 24 months.

Conclusions: This novel bigenic rat model possessing both amyloid and tau pathologies in an aged state is an attractive model for the further investigation of mechanisms by which both proteins effect one another, biomarkers, and elucidation of early disease stages.

Aknowledgements | JTE is the recipient of a 2019-20 FRQS doctoral training fellowship as well as the FSB miller memorial fellowship, Hugh E. Burke fellowship awarded through the Faculty of Medicine at McGill University. The project was CIHR-funded (ACC)

Jennifer Chen

Role of the transcription elongation factor Rtf1 in response to replication stress

Jennifer J. Chen, Ziad El-Hajj, Viviane Pagé, Rodrigo Reyes, Jason C. Tanny

RNA polymerase II (RNAPII) mediated transcription is directly coupled to co-transcriptional processes that regulate numerous aspects of genomic function such as post translational histone modifications and RNA processing. Proper regulation of these processes across the stages of transcription (initiation, elongation and termination) requires spatially and temporally precise recruitment of specific factors to specific chromatin locations. The different stages of RNAPII transcription are marked by changes in the phosphorylation state of the C-terminal domain (CTD) repeats of Rpb1, the largest subunit of RNAPII. Rtf1 is a conserved elongation factor that stimulates RNAPII elongation rate and is required for the deposition of co-transcriptional histone modifications, including H2B monoubiquitylation and H3 lysine 4 methylation. Previous studies have defined Rtf1 interactions that govern these functions, but it remains unclear how they come into play in the regulation of chromatin and transcription in vivo. To address this, we used a genetic approach in the model eukaryote S. pombe and found that Rtf1 mutants were resistant to replication stress caused by treatment with hydroxyurea (HU). Therefore, we hypothesized that Rtf1 (and by extension, co-transcriptional histone modifications) play a particularly important role in regulating the transcription of genes involved in specific stress responses. We investigated potential mechanisms for HU resistance and discovered a role for Rtf1 in governing DNA damage checkpoints. Unexpectedly, we identified multiple mutations in the RNAPII CTD that lead to HU resistance, suggesting potential overlapping functions for Rtf1 with the RNAPII CTD. Finally, we found that Rtf1 mutants significantly blunt the transcriptional response to HU. Using this HU resistance phenotype, we illuminate novel Rtf1 functions in regulating cellular responses to replication stress.

Aknowledgements | This work was supported by the Canadian Institutes for Health Research and the Natural Sciences and Engineering Research Council of Canada. J.J.C is supported by a doctoral fellowship from the Fonds de recherche santé Quebec.

Yanis Inglebert

Synaptopodin, an actin-associated protein, is required for synaptic plasticity at CA3-CA1 synapses

Yanis Inglebert, R. Anne McKinney

The actin-associated protein synaptopodin (SP) is mainly found postsynaptically in a subset of mature dendritic spines. It was reported that lack of SP is associated with reduced learning and defective long-term potentiation (LTP) induced by high frequencies stimulation. But, to date, it is not clear if SP is a requirement for LTP induced by Spike Timing-Dependent Plasticity (STDP) or timing-dependent LTP (t-LTP). t-LTP is induced when synaptic activity is followed backpropagating action potentials (bAPs) (positive timing, $\Delta t > 0ms$) in the post-synaptic cell. Timing-dependent long-term synaptic depression (t-LTD) is expressed when synaptic activity is repeatedly preceded bAPs (negative timing, $\Delta t < 0ms$). To function, STDP required calcium (Ca^{2+}) elevation in the post-synaptic spine. Interestingly, SP is often associated with the spine apparatus organelle a source of intracellular Ca^{2+} stores. We hypothesized that SP is required for normal STDP at CA3-CA1 synapses. We recorded CA1 pyramidal cell in whole-cell patch-clamp from acute hippocampal slices issued from adult (P14-P20) C57Bl/6 (WT) and SP-deficient (SPKO) mice. Following a classical t-LTP and t-LTD protocol, our results showed a normal STDP curve in WT mice, with t-LTP for positive timing and t-LTD for negative timing. On the contrary, SPKO mice revealed a lack of t-LTP for positive timing but t-LTD instead. But, increasing pairing frequency from 0.3 Hz to 5 or 10 Hz can recover a normal t-LTP in SPKO mice. Together, our results highlight the importance of SP in STDP at CA3-CA1 synapses.

Aknowledgements | CIHR, NSERC, The Norman Zavalkoff Family Foundation

Individual differences in ultrasonic vocalizations and sucrose preference: temporally stable and uncorrelated in adult rats

Adithi Sundarakrishnan and Paul B.S. Clarke

Sucrose preference (SP) is a widely used measure of anhedonia in rat models of depression, yet depressed patients do not reliably show an analogous deficit. As an alternative affect-related measure, adult rat ultrasonic vocalizations (USVs) are attracting interest, but it is unclear whether SP and USVs provide independent measures. Here, we have assessed whether SP and USV emission are correlated under basal (i.e., non-evoked) conditions. To this end, 24 male Long-Evans rats were tested daily for 24 days, with alternating SP tests and USV recordings; after a 3-month hiatus, USV emission was re-evaluated for 6 more daily tests. Throughout, SP was measured in simultaneous two-bottle choice tests, and USVs were recorded in an open field. The main measures were: SP, 50-kHz call rate, and relative prevalence of trill and flat call subtypes. These measures showed temporally-stable individual differences across the initial 24-day testing period, and at the 3-month follow-up. Correlational analysis revealed no significant relationships between SP and the three main USV measures. Rats differed consistently in their 50-kHz call rates and also in their 50-kHz call profiles (i.e., the relative prevalence of 14 call subtypes); most rats preferentially emitted either trill or flat calls. Several inter-call subtype associations were detected, including a strong negative relationship between the relative prevalence of flat and trill calls. In terms of relative subtype prevalence, call rate was only correlated with the short call subtype. In conclusion, adult rats exhibited temporally-stable individual differences over weeks (SP) or months (USVs) of testing. This trait-like stability helped to reveal a lack of relationship between SP and the USV-related variables under basal conditions. Sucrose preference and USVs may therefore capture different affect-related constructs, of possible relevance to animal models of depression.

Aknowledgements | Acknowledgements: I would like to thank all my lab members for their support and feedback. Funding: Supported by a Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant.

Daniel Mark Sapozhnikov

Enzyme-free targeted DNA demethylation in mammalian cells

Daniel Sapozhnikov, Moshe Szyf

Despite extensive demonstrations of the association between DNA methylation and gene expression across hundreds of phenotypes, the causality of this relationship remains unresolved. In this work we, demonstrat that experimental confounds preclude resolution of this question with existing strategies, including recently developed CRISPR/dCas9 and TET-based epigenetic editors. Instead, we demonstrate a highly effective method using only guide-RNA-directed nuclease-dead Cas9 to physically block DNA methylation at specific targets in the absence of any confounding flexibly-tethered enzyme, thereby enabling the examination of the role of DNA demethylation in living cells. Using this method, we probe a small number of inducible promoters and find the effect of DNA demethylation to be small, while demethylation of CpG-rich FMR1 produces larger changes in gene expression. We show that this method has no off-targets and results in genome-wide DNA methylation profiles that are more similar to untreated cells than dCas9-TET-based methods.

Aknowledgements | We thank CIHR, NSERC, and the Faculty of Medicine of McGill University for providing funding.

Etienne Billard

5-HT2AR/AMPAR Heteroreceptor Complex As A Mediator Of LSD Action.

Etienne Billard, Robert Rizk, Darlaine Pétrin, Gabriella Gobbi, Terry Hébert

The administration of psychedelic compounds in controlled clinical settings leads to antidepressant and anxiolytic effects, accompanied by changes in brain activity and connectivity. Although lysergic acid diethylamide (LSD) is a potent biased agonists of the G protein-coupled receptor (GPCR) serotonin 2A (5-HT2A), it also modulates the glutamatergic system, notably through the receptor AMPAR. LSD enhances social behavior and has anxiolytic effects in rodents by promoting synaptic plasticity via an AMPAR-dependent mechanisms, while in layer V pyramidal neurons psychedelics induce phosphorylation of AMPAR GluA2 subunit and promote its internalization. As several GPCRs (dopamine D2 and β2-adrenergic) can form supramolecular complexes with AMPAR, our hypothesis is that LSD-mediated AMPAR activation can occur via an oligomeric AMPAR/5-HT2AR complex.

To demonstrate physical interactions between AMPAR and 5-HT2AR we used co-immunoprecipitation in HEK-293 cells and proximity ligation assays in rat primary cortical neurons. To characterise the unique signalling fingerprint of this heteroreceptor complex we used RET-based biosensors.

We have demonstrated that 5-HT2AR and AMPAR can be co-immunoprecipitated, thus being part of a complex. Proximity ligation assays confirmed such physical interaction in neuron. Co-transfection of AMPAR with 5-HT2AR did not impact Gq activation following treatment with LSD, but decreased efficacy for β-arrestin2 recruitment, demonstrating the impact of AMPAR in biasing 5-HT2AR signaling through lateral allostery.

Our in vitro data support the existence of a 5-HT2AR/AMPAR hetero-oligomers and suggests further research in primary neuronal culture and in vivo is required. We believe that the 5-HT2AR/AMPAR could be regarded as a new therapeutic target for treating mental illness.

Aknowledgements | Many thanks to Domo for his steady mentorship! Thanks to the funding : RQSHA, MITACS, Diamond Therapeutics

3-Minute Flash Talks

Format

The 3-Minute Flash Talk presentation is intended to give graduate students the opportunity to give an overview of their research project. This is not a typical 3-Minute Thesis as the audience is a scientific community, and specifics on one's research project are welcome.

Duration: 3 minutes.

Question period: N/A.

Presentation format: Powerpoint. Recommended: 3 slides, although not required. Can be static or animated slides.

Schedule

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

Andrew Bayne, PhD – Trempe Lab
Evan Buddle, Incoming MSc – Bernard Lab
Rebecca Cummer, PhD – Castagner Lab
Bruktawit Maru, PhD – McKeague Lab
Justin Meneses, PhD – Castagner Lab
Jasmine Phénix, PhD – Münter Lab
Reilly Pidgeon, PhD – Castagner Lab

Vote | Flash Talk Award
(click in red box or scan QR code)

Andrew Bayne

Deciphering mitochondrial targeting sequences with bioinformatics and in vitro approaches

Andrew Bayne, Jing Dong, Saeid Amiri, Sali Farhan, Jean-François Trempe

The coordinated localization and import of proteins into mitochondria is an essential process that ensures mitochondrial homeostasis and consequently cell survival. Most mitochondrial proteins are localized and imported via N-terminal mitochondrial targeting sequences (MTSs), which interact with translocases of the outer membrane and are removed in the matrix by the mitochondrial processing peptidase (MPP). The recent discovery of internal MTSs – those which are distributed throughout a protein and act as import regulators or secondary MPP cleavage sites – has expanded the role of MTSs and MPP beyond their canonical N-terminal pathways. Still, the processing of MTSs remains poorly characterized, both from genetic and biochemical perspectives. To address these gaps, we developed a bioinformatics platform (MTSviewer) to visualize clinically relevant MTS mutations and facilitate their investigation in vitro. This flash talk will highlight MTSviewer and our recent work on the import and processing of PINK1 – a cryptic MPP substrate implicated in Parkinson's disease.

Evan Buddle

Conservation of mechanisms regulating *Tgfbr3l/TGFBR3L* transcription

Evan R. S. Buddle, Claire Lin, Daniel J. Bernard

Follicle-stimulating hormone (FSH) regulates gonadal physiology and reproduction. Inhibins and activins, ligands in the TGFβ family, suppress and stimulate FSH production by pituitary gonadotrope cells. Activins bind and signal via complexes of type II and I receptor serine/threonine kinases. Inhibins are competitive antagonists that block activin signaling by binding and sequestering type II activin receptors. Inhibins bind these receptors with high affinity or avidity in the presence of co-receptors. Two inhibin co-receptors have been described, TGFβ type III receptor (TGFBR3, or betaglycan) and TGFBR3-like (TGFBR3L). Betaglycan is ubiquitously expressed. In contrast, TGFBR3L is only expressed in gonadotropes. Our lab previously established that the transcription factors steroidogenic factor 1 (SF1, product of the *Nr5a1* gene) and early growth response 1 (EGR1) regulate murine *Tgfbr3l* transcription by binding to *cis*-elements in the proximal promoter. Here, we report that this mechanism is conserved in humans. Endogenous SF1 from homologous gonadotrope-like cells bind to two *cis*-elements in the human *TGFBR3L* promoter. In heterologous cells, exogenously expressed SF1 and EGR1 independently and synergistically stimulate human *TGFBR3L* promoter-reporter activity via their defined *cis*-elements. In homologous gonadotrope-like cells, mutation of these *cis*-elements or knockdown of endogenous *Nr5a1* or *Egr1* reduces *TGFBR3L* promoter-reporter activity. In summary, our results indicate that SF1 and EGR1 regulation of *TGFBR3L* transcription is conserved in humans and explains, at least in part, gonadotrope-specific expression of the gene.

Aknowledgements | This work was supported by the Dr. Chen & Li Biomedical Research Bursary to ERSB, CIHR CGSM and Dr. Samuel Solomon Fellowship in Endocrinology to CL, and CIHR PJT-162343 to DJB.

Rebecca Cummer

Disarming *Clostridioides difficile*

Rebecca Cummer, Bastien Castagner

Clostridioides difficile (C. diff) is a bacterium that causes the most prevalent form of hospital-acquired infection. This infection can cause symptoms varying from diarrhea to life-threatening inflammation of the colon. C. diff is considered one of the 5 most urgent resistant pathogens by the U.S Center for Disease Control and Prevention. The most prevalent treatments for C. diff infections are antibiotics. However, antibiotics kill bacteria in the intestine that prevent C. diff from colonizing the intestine, resulting in high rates of disease relapse. As a result, there is a need for new treatments against C. diff infections. One treatment strategy would be to target toxins released by C. diff in the intestine. The toxins make an ideal drug target as they are the primary cause of symptoms. At present there are no small molecule drugs that target these toxins, neither on the market nor in clinical testing. We propose to develop a small molecule drug that disassembles the toxins, rendering them inactive, before they provoke disease. Our lab has previously shown the efficacy of small molecules capable of inactivating the C. diff toxin. We are currently optimizing our molecules by probing the structure activity relationship between the small molecule and toxin. This drug design tactic could provide a much-needed alternative to antibiotics for treating C. diff infections.

Bruktawit Maru

Identification and Targeting of Predictive Biomarkers of Cytarabine Efficacy in Acute Myeloid Leukemia

Bruktawit Maru, Nathan Luedtke, Maureen McKeague

Acute Myeloid Leukemia (AML) is a devastating disease with approximately 30% five-year overall survival. It is a highly heterogeneous disease that is characterized by abnormal differentiation and clonal proliferation of immature myeloid cells at various stages of maturation. Although we understand the highly heterogeneous nature of AML, the current front-line induction therapy for most AML patients remains a ‘7+3’ regimen with 7 days of cytarabine and 3 days of anthracycline treatment. After more than 50 years of its widespread use, the mechanism(s) responsible for cytarabine’s selective killing of white blood cells is poorly understood. Therefore, we are assessing the mechanism(s) of death induced by standard, first-line curative treatments of AML within different AML subtypes. Furthermore, we are identifying predictive biomarkers of cytarabine efficacy to better stratify patients for "salvage-like” therapy. Specifically, we are studying transcriptional biomarkers involved in the cytarabine-mediated cell death mechanism(s) and developing RNA therapeutics to re-sensitize AML to cytarabine. If successful, we envision a personalized medicine approach where patients with low sensitivity to cytarabine are identified at the time of diagnosis, and a suitable therapeutic oligonucleotide is prescribed as an adjuvant therapy that increases the efficacy of cytarabine.

Justin Meneses

Development of novel HIV maturation inhibitors

Justin Meneses, Robert Dick, Bastien Castagner

Human immunodeficiency virus (HIV) is a virus that attacks human immune cells, leaving infected individuals vulnerable to other common pathogens. As of 2020, 26 million HIV patients were receiving the standard of care treatment: highly active antiretroviral therapy. While this therapy has largely been effective at treating HIV infection, there is a continuing need for new long-acting drugs to overcome problems with patient tolerability, dose adherence, and emerging antiretroviral drug resistance. To this end, maturation inhibitors (MIs) are a new class of antiretroviral that are distinguished by their unique target and mechanism of action. These drugs work by stabilizing the capsid-spacer peptide 1 (CA-SP1) region of the immature polyprotein Gag and preventing viral protease mediated cleavage at this site. Recently, inositol hexakisphosphate (IP6) has been characterized as a structural co-factor in HIV that is critical to viral maturation and replication. The IP6 binding pore is located directly above the first-in-class MI, bevirimat (BVM) in the immature Gag hexamer. However, it remains unclear if the IP6 binding pocket can be exploited by novel MIs. To answer this question, we aim to design, synthesize, and evaluate novel MIs based on BVM and inositol phosphates. We hypothesize that expanding MIs into the IP6 binding pore in order to displace IP6 or mimic its function without allowing its repackaging can be an effective approach to inhibiting HIV maturation. Further, simultaneous inhibition of CA-SP1 cleavage and IP6 packaging could provide dual inhibitory mechanisms and lead to more potent MIs that are less susceptible to the evolution of resistance. The development of dual mechanism maturation inhibitors may help solve some of the issues currently plaguing HIV therapy.

Jasmine Phénix

The Cholesteryl Ester Transfer Protein in Alzheimer's Disease

Jasmine Phenix, Lisa Münter

Alzheimer's disease (AD) is the most common form of dementia and affects around 35 million individuals worldwide with currently no cure. Several studies showed an association between high levels of plasma pro-atherogenic low-density lipoproteins (LDL) cholesterol and an increased risk of developing AD. The cholesteryl ester transfer protein (CETP) is a cholesterol transporter whose activity leads to more cholesteryl esters in LDL particles, and thus higher levels of LDL cholesterol in the blood. Recent epidemiological studies have shown an association between CETP activity and the risk of developing AD. My project proposes a new perspective on AD research by investigating CETP as main pharmacological target. To examine the effects of CETP inhibition on AD pathology, mice from four different genotypes: wildtype mice, human CETP transgenic mice (hCETP), human APP transgenic mice (hAPP), and hAPP/hCETP double transgenic mice were administered CETP inhibitor evacetrapib or vehicle daily at 2 months of age for 10 weeks. At 5 months of age, hAPP mice were cognitively impaired both in the evacetrapib and vehicle condition. Interestingly, both hCETP and double transgenic mice were cognitively impaired in females only, which could be rescued by evacetrapib indicating a role of CETP in the early stages of AD. Cognitive rescue also significantly correlated with serum HDL (positive) and LDL levels (negative). Moreover, using ELISA to quantify Aβ species, we demonstrated that levels of Aβ peptides do not correlate with cognitive impairment, which contrasts with the amyloid hypothesis. Most interestingly, hCETP and hAPP/CETP mice treated with evacetrapib showed a significant increase in brain cholesterol as compared to controls, which could indicate that cerebral cholesterol synthesis is important in good cognition. As such, we anticipate the activity of CETP to be a contributing factor in worsening the disease, while its pharmacological inhibition may ameliorate AD pathology.

Aknowledgements | Dr. Claudio Cuello for his hAPP transgenic mice. Dr. Noosha Yousefpour for training.

Reilly Pidgeon

Modulating the gut microbiota to fight cancer

Reilly Pidgeon, Meriem Messaoudene, Corentin Richard, André Marette, Bertrand Routy, Bastien Castagner

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of cancers; however, long-lasting tumor control only occurs in a minority of patients. The composition and function of the gut microbiota is a key immune mediator of ICI activity. Therefore, microbiota manipulation is a promising therapeutic approach to improve ICI efficacy. The gut microbiota is primarily shaped by environmental factors rather than host genetics. Microbiota composition and function can be manipulated in two major ways: supplementation of exogenous bacteria (probiotics or fecal microbiota transplants) or modification of the endogenous microbiota (dietary prebiotics, xenobiotics, or antibiotics). In the context of cancer immunotherapy, prebiotics that are utilized by host microorganisms are preferred as they do not require colonization of exogenous bacteria within an established gut community. Multiple dietary sources of prebiotic compounds are being investigated in combination with cancer immunotherapy. We have found that the prebiotic-rich camu-camu berry can circumvent ICI resistance in mice in a microbiota-dependent manner. A drawback of working with whole fruits and their extracts is batch-to-batch variability caused by geographical location, cultivation conditions, ripeness, and fruit processing conditions. Therefore, we purified and iteratively screened camu-camu fractions in mouse models of cancer. Using this screening technique, we identified castalagin as the microbiota-dependent bioactive compound in camu-camu. Similar to camu-camu, castalagin gavage in tumor-bearing mice led to a significant change in microbiome composition and metabolism and led to CD8+ T-cell-dependent anti-tumor activity. Altogether, we discovered a defined prebiotic compound that improves cancer immunotherapy efficacy. Future work will address the functional changes caused by castalagin and its metabolites on growth, gene expression, and community dynamics of gut bacteria.

Aknowledgements | Weston Family Foundation, Canadian Institutes of Health Research, and Fonds de recherche du Québec – Santé

Poster Presentations

Format

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

Duration: 45 minutes (each session). Make sure to have a 3 minute elevator pitch prepared for the judges.

Question period: Conversational.

Schedule

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

Poster	Presenter
1	Quentin Bonomo
3	Jose Antonio Vazquez Coba
5	Jacob Blaney
7	Emma Paulus
9	Rita Gloria Ihirwe
11	Grace Mazarura
13	Lucia Wang
15	Ariane Lismer
17	Quazi Islam
19	Zixuan Li
21	Jamie Mustian
23	Emily Wilson
25	Garvit Bhatt
27	Peiyou Wu
29	Lucas Marques
31	Xiaotong (Vicky) Wang
33	Heather Fice
35	Anne-Sophie Pépin
37	Maja Beus
39	Amelia Kulle
41	Ai Liu

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

Poster	Presenter
2	Juliana Dallagnol
4	Patrick-Brian Bielawski
6	Sofia Paoli
8	Cara Hawey
10	Olivia Kovecses
12	Tara Shomali
14	Ylauna Penalva
16	Braeden Giles
18	Yangfan Jin
20	Hanan Mohammad
22	Ali Shariat-Panahi
24	Dongwei (Oscar) Yu
26	Seline Vancolen
28	Tapan Agnihotri
30	Louis-Charles Masson
32	Kyla Bourque
34	Sarah MacKinnon
36	Ali Nejatie
38	Lama Iskandarani
40	Luisina Ongaro
42	Benjamin Kannel

Quentin Bonomo

Characterization of the McGill-R955-hTau rat model of human-like tauopathy.

Quentin Bonomo, Sonia Do Carmo, Joshua Emmerson, A. Claudio Cuello

Recently, the focus of Alzheimer Disease (AD) therapeutics has increasingly shifted toward tau protein, which is a more effective predictor of neurodegeneration and cognitive impairment compared with amyloid-β. Exploration of tau-targeted therapies raises the fundamental need of generating animal models of tauopathy with high translational value to humans. Tau-based models, mostly in mouse, provided invaluable insights into the mechanism involved in AD pathology. However, most models fail to show significant neurodegeneration, a crucial hallmark of AD.

To overcome these limitations, our lab generated the McGill-R955-hTau rat model of human-like tauopathy. This model overexpresses the longest isoform (2N4R) of human tau bearing the P301S mutation, known to cause frontotemporal dementia. Of relevance to AD pathology, rats display all six human tau isoforms, unlike mice, and the rat endogenous ApoE protein has a high homology with the human ApoE4 protein.

Our overarching goal is to characterize a rat model that recapitulates key features of human AD-like tauopathy, along with behavioral deficits and neurodegeneration. Here, we tested the cognitive performance of 15 months-old McGill-R955-hTau transgenic rats. At this time-point, this model displays abnormal tau hyperphosphorylation in the absence of tau aggregation and neuronal loss.

To test their memory and learning capabilities, rats were subjected to the open field, novel object recognition, Y-maze, social interaction and Morris water maze tests. Assessment of cognition shows no significant difference in performance between homozygous transgenic rats and wild-type littermates at 15 months of age. Examination of tau pathology is in progress.

Our new rat model should faithfully recapitulate the disease progression reported in human patients, offering a valuable model to predict the clinical efficacy of therapeutic interventions in halting or even reversing tau pathology in AD and other tauopathies.

Aknowledgements | CIHR

Juliana Dallagnol

Synthesis of prodrug angiotensin II derivatives selectively targeting intracellular G protein-coupled receptors as promising treatments for heart disease

Juliana C. C. Dallagnol, Mikhail Volkovich, David Chatenet, Bruce G. Allen, Terence Hébert

Heart disease remains the leading cause of death globally over the last 15 years, despite a number of drugs being available to either control risk factors or improve cardiac function in heart failure patients. Even though the available medications temporarily improve patients' quality of life; these drugs are not able to prevent heart muscle remodeling and loss of function over time. Consequently, their long-term efficacy is limited. Our work explores the therapeutic potential of intracellular-targeted angiotensin (Ang II) analogs (compounds that selectively activate intracellular localized angiotensin receptors - iATRs). Using photoreleasable-Ang II, our group demonstrated that the selective activation of iATRs evokes distinct responses compared to activation of cell surface receptor counterparts. Here we showed that different Ang II analogs (b-arrestin or G protein biased ligands), similarly targeting the intracellular pool of receptors, can stimulate collagen secretion in primary cardiac fibroblasts in different manners. While b-arrestin-biased cTRV027 and cSI do not promote collagen secretion after UV exposure; the G protein-biased cTRV055 and cTRV056 do. Better understanding these non-canonical signaling responses may help the development of drugs that prevent heart remodeling thus advancing toward better therapeutic options.

Glucose metabolism as a function of ApoE genotype?

Jose-Antonio Vazquez-Coba, Jasmine Phenix , Ylauna Penalva , Scott De-Vito, Lisa Münter

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common cause of dementia worldwide, primarily affecting the elderly. AD patients show severely diminished glucose uptake into the brain but at the cellular level, it is less clear. Astrocytes glial cells in the nervous system and key mediators for maintaining homeostasis, quickly responding to several stress factors, like hypoxia. Additionally, astrocytes are the main producers of the apolipoprotein E (ApoE), its isoform ε4 is the main genetic risk factor for sporadic AD, while the isoform ε3 is regarded neutral with respect to AD risk. However, our understanding of ApoE's function in the brain is limited. In addition, GLUT1 is the main glucose transporter in astrocytes and its expression levels have never been assessed as a function of ApoE genotype to our knowledge. Our aim is to understand the differences in the glucose metabolism in astrocytes depending in the ApoE isoform express. We hypothesized that the ApoE4 expressing astrocytes will show an impaired glucose metabolism in comparison to the other isoforms.Immortalized ApoE3/E4-expressing mouse astrocytes on cell culture were used. An increase in relative protein expression of GLUT1 levels was found in ApoE4 in contrast with the other isoforms. GLUT1-expression at the cell surface by flow cytometry was found to be increased in apoE4 expressing mouse astrocytes too. However, despite this protein increase, a glucose uptake assay revealed no significant changes in glucose uptake between the difference isoforms. ApoE4 expressing mouse astrocytes showed increased expression of GLUT1 in total protein cell lysates and flow cytometry. Despite higher GLUT1 expression, glucose uptake remained unaffected in comparison to the other isoforms. Further analyses will need to be done to characterize this phenotype seen and to corroborate if the ApoE isoform expressed has a direct effect in glucose metabolism.

Aknowledgements | Integrated Program in Neuroscience

Central and Peripheral Macrophage Response to Cellsomes in Ischemic Stroke

Patrick-Brian Bielawski, Issan Zhang, Francisco Campos Perez, Ester Perez-Polo, and Dusica Maysinger

Ischemic stroke remains a leading cause of death and disability around the world. Although many attempts have been made to develop novel therapeutics, tissue plasminogen activator (tPA) remains the only approved drug, and not without its own share of limitations. Encapsulating tPA in a biomimetic nanocarrier (cellsome) derived from platelets would increase tPA's localisation to the clot site and protect tPA from degradation. A major contributor to the progression of neuronal death and inflammation following stroke is high-mobility group box 1 (HMGB1), an alarmin and redox-sensitive damage associated molecular pattern. Both the acetylated and oxidised forms of HMGB1 can activate immune cells to further release HMGB1 and pro-inflammatory cytokines. The objective of our study is to determine if tPA-loaded cellsomes have a differential effect on human microglia and macrophages compared to free tPA. We assessed cellular viability, intracellular reactive oxygen species (ROS) levels, and levels of acetylated (acHMGB1) in the cytosol. We found that tPA, empty cellsomes, and tPA-loaded cellsomes had no detrimental effects on cell viability up to 20 µg/mL nor did tPA increase apoptosis or necrosis in microglia cells. We found that acHMGB1 levels in microglia did not significantly increase under higher tPA concentrations, whereas ROS levels were found to be increased in a dose dependent manner. tPA-loaded cellsomes demonstrated a slight decrease in ROS levels compared to tPA alone at 0.1 µg/mL. These results suggest that cellsomes loaded with tPA do not negatively affect microglia and macrophage viability, and do not increase reactive oxygen species levels compared to free tPA. Future experiments will further expand on the modulation of acHMGB1 levels and localisation under cellsome-loaded tPA treatment.

Aknowledgements | Funded by EuroNanoMed III and the National Sciences and Engineering Research Council of Canada (NSERC).

Investigating the interaction between the SWI/SNF chromatin remodeling complex and Gβ1 signaling in cardiac fibrosis

Jacob Blaney, Darlaine Pétrin, Ryan Martin, Celia Bouazza, Jason C. Tanny, Terence E. Hébert

The formation of fibrotic tissue, mediated by cardiac fibroblasts, is an important repair and maintenance process that becomes dysregulated in the development of heart failure. During prolonged hypertensive or ischemic events, fibroblast stimulation by angiotensin II (Ang II) promotes increased secretion of extracellular matrix proteins, accelerated cell mobility, and production of pro-fibrotic signalling molecules. We have found a non-canonical, nuclear interaction between Gβ1 and both RNA polymerase II and the SMARCA4 subunit of the SWI/SNF chromatin remodelling complex, suggesting direct G-protein regulation of the fibrosis transcriptional program. This leads us to hypothesize that, by interacting with SMARCA4, Gβ1 acts as a transient brake that suppresses the fibrotic response until sufficient stimulation accumulates. To test this, we will 1) determine the role of SMARCA4 and Gβ1 on common fibrotic processes, 2) survey transcriptomic changes in fibroblasts where SMARCA4 and Gβ1 have been knocked down, and 3) use chromatin occupancy to assess the functional relationship between Gβ1 and SMARCA4 during the fibrotic response. To assess changes in the fibrotic response following knockdown of SMARCA4 or Gβ1 in rat neonatal cardiac fibroblasts (RNCFs), wound closure rate will be quantified by scratch assay. Then, RNA sequencing and pathway analysis will be performed to establish interaction patterns between siRNA and agonist-treated conditions. Finally, gene loci where Gβ1/SMARCA4 interacts will be identified using CUT&RUN. Scratch assay results showed that RNCF migration is dependent on media serum supplementation. When treated with 1 μM Ang II, RNCFs showed varying changes in migration rates, but 30-minute pre-treatment with 10 nM losartan consistently slowed migration. Further optimization of siRNA transfection is underway to investigate the effects of Gβ1/SMARCA4 knockdown on the fibrotic response and to evaluate further transcriptional consequences.

Aknowledgements | This project was funded by CIHR and NSERC.

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

Sofia Paoli, David H. Eidelman and Carolyn J. Baglole

Electronic (e-) cigarettes are battery-powered devices that produce an aerosol by heating up a liquid composed of nicotine, flavouring agents, propylene glycol and vegetable glycerin (PGVG). In recent years, e-cigarettes have gained popularity among adolescents, raising concerns over the potential adverse effects in youth with respiratory diseases, such as allergic asthma. We previously showed that an acute exposure to mango and mint flavoured aerosols increased lung neutrophils and upregulated the expression of pro-inflammatory cytokines in the lungs. In this study, we aimed to determine how a sub-chronic exposure to a flavoured aerosol affected airway and pulmonary inflammation in a murine model of allergic asthma. Male and female C57BL/6 mice were exposed to a mint flavoured aerosol containing nicotine for 14 days. The control groups were exposed to air only or PGVG only. Then, three mice from each group were euthanized immediately after the last exposure to characterize immediate effects. The remaining mice from each group were sensitized and challenged with PBS or ovalbumin (OVA) to induce inflammation, a key feature of an asthmatic phenotype (Days 0, 7, 14-16). We collected the bronchoalveolar lavage (BAL) fluid and the lung tissue to characterize inflammation via flow cytometry and measure gene transcription changes via RT-qPCR. The mice exposed to e-cigarettes only had significantly increased mRNA levels of several pro-inflammatory and oxidative stress response genes. Surprisingly, these genes were also upregulated in mice exposed to PGVG and treated with PBS, suggesting that exposure to aerosolized PGVG may have harmful effects. Immunophenotyping of BAL immune cells revealed infiltration of neutrophils in the BAL of OVA-treated mice, which was slightly higher in the mice exposed to JUUL. Overall, these results indicate that e-cigarettes cause inflammation and oxidative stress in the lungs, and may pose a serious threat to youth with respiratory diseases.

Emma Paulus

Biased Agonism at the Dopamine 1 Receptor: In Vitro

Emma Paulus, Jace Jones-Tabah, Paul B.S. Clarke, Terry Hebert

The Dopamine 1 receptor (D1R), a member of the G-protein coupled receptor family, has been explored as a therapeutic target for Parkinson's disease. When a biased agonist binds to a D1R, it preferentially stimulates a subset of intracellular signaling pathways. To date, most studies on biased agonism have been based in heterologous systems such as HEK-293 cells. We aim to investigate biased signaling at the D1R in HEK-293 cells and in more physiologically-relevant cells--rat neonatal primary striatal neurons (rPSN)--in order to establish which aspects of GPCR signaling can be effectively modeled in heterologous cells, and where they need to be replaced by primary cells. In both cell types, a panel of D1R agonists will be used to examine signaling events downstream of the D1R. HEK-293 cells will be transiently transfected with the D1R and relevant biosensors to measure effectors of the D1R, and cells will be assayed on 96-well plates to assay biosensor outputs. In rPSN, we will use an existing repertoire of FRET-based biosensors to quantify effectors of the D1R and signaling will be assayed in 96-well plates using high-content microscopy. We expect the D1R agonists that are full, partial, or biased agonists to display full, partial, or biased agonism, respectively, in both the HEK-293 cell lines and in the primary neurons. We anticipate that differences in signaling bias between the two cell types will be observed at the level of receptor-distal events. The differential effects of the D1R agonists between HEK-293 cells and rPSN will help establish which aspects of GPCR signaling can be effectively modeled in heterologous cells, and where they need to be replaced by primary cells. Our findings will elucidate the extent to which biased agonism is translatable from in vitro to in vivo settings.

Aknowledgements | CIHR, ABIF

Cara Hawey

Understanding dilated cardiomyopathy using cardiomyocytes made from patient-derived induced pluripotent stem cells

Cara Hawey, Kyla Bourque, Ida Derish, Jeremy Zwaig, Kashif Khan, Renzo Cecere, Terry Hébert

Dilated cardiomyopathy (DCM) is one of the most common types of heart disease that affect the heart muscle and can progress to serious cardiac consequences such as heart failure and ultimately the need for transplantation. DCM is characterized by dilation of the ventricles and contractile dysfunction, which result in insufficient pumping of oxygenated blood to the rest of the body. DCM can be managed by drugs such as β-blockers; however, morbidity and mortality rates remain unacceptably high, driving the need for a better understanding of the underlying disease mechanisms so that improved and personalized therapeutic options can be developed. Induced pluripotent stem cells (iPSCs) are used to model human diseases in a dish on a patient-to-patient basis. Using iPSC-derived cardiomyocytes (CMs) originating from patient and age-matched healthy volunteers, the goal of this study is to phenotype features underlying DCM to better understand dysregulated contractility. Assays were developed to investigate three main features of iPSC-CMs: sarcomere organization, calcium handling, and functional contractility. Sarcomere organization and calcium handling were measured using a genetically-encoded biosensor, RGECO-TnT, which localizes to the contractile machinery of cardiomyocytes while contractility was evaluated using impedance-based methods. Initial results indicate differences in calcium handling between iPSC-CMs derived from a DCM patient and a healthy volunteer. In addition, differences in calcium and contractility can be detected in response to acute adrenergic stimulation. DCM is a clinically heterogenous disease with many etiologies therefore we expect sarcomere organization, calcium handling, and contractility to vary on a patient-to-patient basis. Differences in phenotype and response to drugs will be used to cluster patients into groups to better advise personalized treatment options.

Aknowledgements | Project funded by the Courtois Foundation and the McGill Faculty of Medicine

Abnormal placental DNA methylation and gene expression associated with assisted reproduction: early detection and effect of folic acid supplementation

Rita Gloria Ihirwe, Josée Martel, Sophia Rahimi and Jacquetta Trasler

Background: Assisted reproductive technologies (ARTs) have been associated with imprinting disorders and adverse placental development in offspring. ARTs are performed during key periods of germ cell and preimplantation development when DNA methylation profiles undergo extensive reprogramming. Dietary folic acid (FA) provides methyl groups for proper DNA methylation establishment and maintenance during embryo development.

Hypothesis: (1) ART alters DNA methylation and gene expression during early placenta development, leading to placental defects later in gestation; and (2) we postulate that folic acid supplementation can rescue these effects.

Methods: Female mice were fed 3 different levels of FA supplementation for 6 weeks prior to natural mating or ART (superovulation, in vitro fertilization, embryo culture and transfer) and throughout gestation. Embryos and placentas were collected at midgestation, and RNA-sequencing performed on placentas from E10.5 embryos. Among the genes affected by ART, 5 genes involved in early placenta development (Phlda2, Igf2, Peg3, EphB2 and L3mbtl1) were chosen for analysis of DNA methylation (Bisulfite pyrosequencing) and gene expression (droplet digital PCR) in placentas from abnormal, developmentally delayed, and normal embryos.

Results: ART induced differential expression of 41 and 28 genes in E10.5 female and male placentas, respectively. In both sexes, ART decreased Phlda2 imprinted gene expression as well as DNA methylation at the Kcnq1ot1 imprinting control region which regulates Phlda2 expression. FA supplementation partially improved DNA methylation and gene expression levels in a sex-specific manner. The epigenetic alterations observed may help explain adverse placental phenotypes detected later in gestation.

Aknowledgements | Supported by CIHR

Drugging Transcription Factors in Acute Myeloid Leukemia using RNA Therapeutics

Olivia Kovecses, Bruktawit Maru, Maureen McKeague

Acute myeloid leukemia (AML) has an average 5-year survival rate of only ~25% despite the use of current front-line therapies. The most prevalent causative mutation of AML is an internal tandem repeat (ITD) in the FMS-like tyrosine kinase 3 (FLT3) receptor. This mutation results in a constitutively active FLT3 receptor, which promotes the survival and proliferation of leukemic cells. Furthermore, constitutive FLT3 signaling impedes the role of essential hematopoietic transcription factors PU.1 and CEBPa. When either CEBPa or PU.1 are downregulated, myeloid precursor cells are unable to differentiate into mature monocytes and granulocytes, resulting in the development of AML. A potential treatment strategy for AML involves targeting this differentiation block. Specifically, leukemic cells can be reprogramed to undergo normal hematopoiesis and differentiate into mature non-dividing myeloid cells by altering the expression of transcription factors. However, with our current repertoire of small molecule drugs and biologics, we can rarely target transcription factors due to their nuclear localization and lack of catalytic activity. Using RNA therapeutics, we hypothesize that these currently “undruggable” targets can be selectively modulated. Specifically, gene expression will be increased using novel small activating RNA (saRNA), consisting of an RNA duplex that selectively targets the promoter sequence of the gene of interest and thereby increases transcription initiation. Currently, the first and only saRNA in clinical trials is the drug “MTL-CEBPA” composed of a CEBPa-targeting saRNA encapsulated in SMARTICLES®, a patented liposomal nanoparticle (Mina Therapeutics Limited). We will examine the utility of MTL-CEBPA in the context of AML while also developing new candidate saRNAs targeting PU.1. CEBPa and PU.1-targeted saRNAs capable of inhibiting proliferation and/or inducing differentiation provide a proof-of-concept for the treatment of AML using RNA drugs.

Aknowledgements | Special thanks to MinaTx, Prof. Nathan Luedtke, Dr. Francois Mercier, Prof. William Pastor & Ishtiaque Hossain, Christian Young, Nathalie Croteau, and the Bernard lab.

Grace Mazarura

Elucidating the role of Angiotensin II signaling in cardiac fibrosis using hiPSCs derived from healthy subjects and DCM patients.

Grace R Mazarura, Kyla Bourque, Darlaine Pétrin, Cara Hawey, Alyson Jiang, Terence E Hébert

Dilated cardiomyopathy (DCM) is a disease of the heart, characterized by dilation of the left ventricle and contractile dysfunction which can ultimately lead to heart failure. Fibrosis is an important process leading to the ventricular remodeling that is characteristic of DCM. The main effector cells responsible for pathological remodeling of the heart are activated cardiac fibroblasts (CFs). CF signaling remains understudied in the context of DCM. Activation of CFs is mediated through the angiotensin II type 1 receptor (AT1R), a G protein-coupled receptor. Angiotensin II (Ang II) signaling via AT1R is thought to be the primary initiator of the fibrotic response which leads to secretion of extracellular matrix proteins and transforming growth factor β1 (TGFβ1), a potent driver of later stages of the fibrotic response. We look to investigate how Ang II signaling may differ between CFs from healthy individuals versus DCM patients. Here, we use human induced pluripotent stem cell-derived cardiac fibroblasts (hiPSC-CFs) to elucidate the signaling events driving the fibrotic response. These cells remain quiescent for longer in culture and provide a more physiologically relevant model to study AT1R signaling because GPCR signaling is species and cell-context dependent. We have optimised an established protocol for differentiating hiPSCs to iPSC-CFs. We then used immunofluorescence and RT-qPCR to examine key tissue-specific genes to confirm differentiation of hiPSC lines into CFs. Following this, we will stimulate cells with Ang II and block the secondary TGFβ1 signaling “arm” of activation and assess the fibrotic response using qPCR to quantify key genes important in fibroblast activation. Lastly, we will use FRET-based biosensors to track PKA and ERK1/2 signaling downstream of Ang II stimulation in both healthy and DCM iPSC-CFs. We hope that having a better grasp on the mechanisms underlying cardiac fibrosis will lead to discovery of better therapies for fibrosis.

Aknowledgements | McGill Internal Funding, CIHR and the Courtois Foundation.

Structure-based design of small-molecule modulators of PINK1

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

PINK1 is a kinase whose loss of function is implicated in autosomal recessive juvenile Parkison's disease. Under normal conditions, PINK1 is responsible for the activation of a mitochondrial quality control (MQC) pathway, which rids the cells of damaged mitochondria. Loss of function mutations in PINK1 halt the MQC pathway and lead to the accumulation of the damaged mitochondria, eventually leading to neuronal loss. Modulation of PINK1 through small molecule compounds could help reinstate the MQC and provide an important research tool in Parkinson's disease (PD) research.

A library of 430 small molecules were screened against Tribolium castaneum PINK1 (TcPINK1) using a Thermal Shift Assay. The TcPINK1 orthologue was used since the Homo sapiens PINK1 (HsPINK1) protein is not readily obtainable at this time. Of the TcPINK1 stabilizers identified, PRT062607 were shown to also inhibit HsPINK1 via an in organello assay. The inhibitor's IC50 was determined using a luminescence-based assay in which the inhibition of ATP consumption by TcPINK1 was measured. PRT062607 was the most potent of the inhibitors found, with an IC50 of 1.6 +/- 0.4 μM. Derivatives with higher potency and affinity towards HsPINK1 are being developed by using PRT062607 as a lead compound and by using HsPINK1 protein derivatives. Two approaches have been taken to design TcPINK1-humanized derivatives. The first approach incorporates the ATP binding domain of HsPINK1 into that of TcPINK1, resulting in the humanized-TcPINK1. The second consists of switching the N-terminal and the C-terminal extension of HsPINK1 for that of TcPINK1 while keeping the rest of the protein intact. The inhibitors are currently being co-crystallized with the humanized-TcPINK1, successful crystallization would reflect the human ATP-binding domain. PRT062607 will serve as lead molecules for the development of PINK1 modulators, a potential therapeutic strategy for PD and provide structural insight on the protein when crystallized.

Aknowledgements | Special thanks to Andrew Bayne and Simon Veyron for their guidance.

Lucia Wang

Developing RNA switch-based tools to investigate regeneration in a vertebrate model

Y. Lucia Wang, Thomas Rynes, Karen Mruk, Maureen McKeague

Rationale: Gaining a better understanding of how healing occurs can help reduce complications after injury. However, regeneration is a complex process involving many pathways. Furthermore, while the response to injury occurs rapidly, the healing process can take months to fully transpire. Understanding the regenerative process requires tools that can be both spatially and temporally controlled to observe early molecular events while also being stable in vivo over long time scales. These tools do not currently exist.

Aims: RNA switches control translation via an mRNA degradation mechanism. We propose the development of a library of small molecule-activated RNA switches which will spatiotemporally control gene expression in a vertebrate model. To achieve this, we have three aims: 1) Produce a library of small-molecule responsive RNA switches, 2) Screen the switch constructs in vitro, and 3) Determine the toxicity of the small molecule targets in zebrafish.

Methods: Aim 1: RNA switch sequences were chosen from the literature and inserted into a zebrafish plasmid at the 3' end of GFP to modulate GFP expression. Aim 2: Switch constructs were transfected into HEK293T cells, treated with small molecule target, and GFP expression was measured. Aim 3: Zebrafish were soaked in increasing concentrations of small molecule for varying amounts of time and their development was observed.

Results: The constructs were confirmed by Sanger sequencing. The theophylline switch shows a 10-fold increase in GFP expression when exposed to 2.5 mM theophylline for 72 hours. However, theophylline affects zebrafish development above a 1mM concentration.

Conclusions: We inserted several switches into a zebrafish plasmid and showed that they are small-molecule responsive. Moving forwards the toxicity results will inform in vitro screening conditions. Once the switches have been validated in vitro, they will be sent to our collaborators to be used, for the first time, in a vertebrate model.

Aknowledgements | Thank you to our collaborators at the Mruk Lab for their zebrafish work and thank you to the NIH for funding this project.

Regulation of cerebral glucose metabolism by APP in the context of Alzheimer's disease

Ylauna Penalva, Lisa Münter

Alzheimer's disease (AD) is a debilitative neurodegenerative disorder characterized by progressive impairments in cognitive functions. It accounts for 50%-60% of all dementia cases and affects 24 million people worldwide. Cerebral glucose hypometabolism, a consistent pathophysiological feature of the disease, is observed well before the onset of early AD symptoms. This defect can be attributed to the decreased expression of the glucose transporter 1 (GLUT1) in AD patients. Indeed, glucose enters the brain predominantly through GLUT1 expressed in endothelial cells lining the cerebral vasculature, and its expression correlates with cerebral glucose uptake. Another disease hallmark is the abundance of Aβ peptides as plaques in the brain. Aβ peptides arise from the sequential proteolytic cleavage of the amyloid precursor protein (APP), a type-I transmembrane protein. Autosomal dominant inherited mutations causatively link APP itself to AD, rendering it imperative to fully understand APP's physiological functions to define the underlying biology of AD. To date, no link has been established between APP and the glucose hypometabolism in AD. Interestingly, our studies in APP knockout mouse embryonic fibroblasts (MEFs) showed that the absence of APP induces a 40% decrease in GLUT1 protein expression and a 3-fold decline in glucose uptake when compared to controls. GLUT1 is majorly regulated through the growth factor responsive PI3K/Akt/mTOR kinase signaling pathway hub. Indeed, we found a 50% decrease in its activation in APP knockout MEFs. To determine the physiological relevance of these findings, we prepared endothelial cells from APP knockout brains and found decreased GLUT1 expression as well as decreased PI3K/Akt/mTOR activation. We propose that APP regulates glucose metabolism by upstream signaling of the PI3K/Akt/mTOR pathway through its potential interaction with growth factors and their receptors.

Aknowledgements | ASRP, CIHR, NSERC, Faculty of Medicine.

Ariane Lismer

Exposure to the toxicant DDT is implicated in paternal epigenetic transmission of developmental disease

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Humans are exposed to a myriad of toxicants that have a multitude of negative health consequences including impacting generations to come. However, the molecular mechanisms mediating this epigenetic inheritance remains almost entirely unexplored in humans. Exposure to the insecticide dichlorodiphenyltrichloroethane (DDT) is linked to birth defects, cancer, and neurodevelopmental delays. Although DDT has been banned world-wide since the 1970s, it is still sanctioned for indoor residual spraying to control malarial disease vectors in Sub-Saharan Africa. The impacts of DDT on human health are not only restricted to regions of use since DDT is transported over long distances to Arctic regions by weather patterns and ocean currents. In this study we studied two geographically diverse DDT-exposed populations, Greenlandic Inuit and South African VhaVenda men, and aimed to determine whether sperm DNAme and chromatin enrichments were associated with levels of DDT exposures and could be implicated in epigenetic inheritance. To identify regions that were altered in DNAme and histone enrichment of exposed men, we used a sperm customized methyl-capture approach and ChIP-seq targeting H3K4me3. We then performed differential and functional analyses to investigate the relationship between DDT-associated alterations in the sperm epigenome and their predicted transmission to the embryo at fertilization. We identified genomic regions with altered DNAme and differential enrichment of H3K4me3 that were dose-like responsive to serum DDE levels. Altered DNAme and H3K4me3 in sperm occurred at regulatory regions involved in fertility, disease, development and neurofunction. A subset of regions with altered sperm DNAme and H3K4me3 occurred at transposable elements and were predicted to persist in the pre-implantation embryo. These findings suggest that DDT exposure in men may negatively impact embryo development and the health of future generations through epigenetic mechanisms.

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Organophosphate esters dysregulate lipid homeostasis in THP-1 macrophages in an LXR independent manner

Braeden Giles, Ayse N. Zengin, Bernard Robaire, Koren Mann

Organophosphate esters (OPEs) are primarily used as flame retardants and plasticizers. Many aspects of OPE toxicity remain unknown; however, two OPEs TPHP and EHDPP have inhibitory binding activity for the nuclear receptor liver X receptor alpha (LXRa) in murine macrophages. LXRa predominantly regulates lipid homeostasis, cholesterol efflux, and autophagy. Inhibition of LXRa increases intracellular lipid content. However, research thus far has focused on single OPEs. Environmentally, OPEs exist as mixtures in which 1) certain OPEs have an increased abundance; and 2) some OPEs have opposing activities. In the present study, we tested the hypothesis that a Canadian household dust-based OPE mixture will alter lipid homeostasis in THP-1 macrophages; and propose that this occurs through inhibition of LXRa. THP-1 human monocytes were chemically differentiated into macrophages before treatment with diluted concentrations of the Canadian household OPE mixture (1x10-4 to 1x10-6) from 4 to 48 hours. Utilizing high-content imaging, we assessed intracellular lipid content and lysosomes. Quantification shows that sustained OPE exposure resulted in a dose-dependent increase in intracellular lipid content and decreases in lysosomes. Targets downstream of LXRa were investigated as a mechanism for intracellular lipid accumulation and alterations in lysosomal activity. THP-1 macrophages were exposed to a 2x10-4 diluted OPE concentration alongside the endogenous LXRa/RXRa ligands 22R-hydroxycholesterol and 9-cis-retinoic acid or synthetic LXRa ligand GW3965. LXRα targets were assessed by qRT-PCR and included genes implicated in lipid efflux (ABCA1), lipogenesis (SREBP1c), and macroautophagy (ATG7, LC3B). Surprisingly, genes downstream of LXRα were unaffected by the OPE mixture. Interestingly, the expected lipid phenotype persists in contrast to previous literature and suggests that the toxicity of this OPE mixture is multifactorial. Supported by CIHR.

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Quazi Sufia Islam

Human R antigen (HuR) controls multiple cellular pathways in TGF-β treated human lung fibroblasts

Quazi Islam, Noof Aloufi, Steven Huang, Dr. David Eidelman, Dr. Carolyn Baglole

Introduction: Idiopathic Pulmonary Fibrosis (IPF), a chronic and irreversible lung disease, is characterized by permanent scarring of the lung, resulting in death from respiratory failure. The main driver of chronic scarring of the lung occurs because of the accumulation of myofibroblasts caused by transforming growth factor β (TGF-β) production of excessive amounts of extracellular matrix (ECM). Recently in our lab, we showed that human antigen R (HuR), an RNA binding protein, drives the differentiation of lung fibroblasts into myofibroblasts upon its cytoplasmic localization in response to TGF-β, along with ECM deposition. Correspondingly, in the lung of IPF patients, HuR was predominantly cytoplasmic, suggestive of its activation. I, therefore, hypothesize that the function of HuR is dysregulated in IPF lung fibroblasts.

Methods: The expression and localization of HuR after exposure to TGF-β in normal and IPF human lung fibroblasts (HLF) was measured by Western Blot, RT-qPCR & IF. HuR knockdown by siRNA followed by mass spectrometry (MS)-based proteomics was performed to comprehensively evaluate the changes in HuR-regulated proteins under myofibroblast differentiating conditions.

Results: There was no significant difference in the total level of HuR between normal and IPF HLFs upon exposure to TGF-β. HuR translocated to the cytoplasm similarly in response to TGF-β in both normal and IPF HLFs. However, proteomic data showed a significant differential role for HuR in response to TGF-β, with GO enrichment highlighting its involvement in established pathways implicated in IPF, including cellular response to stimulus, protein SUMOylation, biological processes in extracellular structure and matrix organization.

Conclusions: This research provides novel information on the critical function of HuR in lung fibroblast differentiation. Thus, HuR may serve as a mechanism through which new therapies could be directed.

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Regulation of follicle-stimulating hormone β subunit transcription by newly discovered enhancers

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Follicle-stimulating hormone (FSH), a dimeric glycoprotein produced by pituitary gonadotrope cells, is an essential regulator of gonadal function. FSHβ subunit (*Fshb*) transcription is rate-limiting in producing the mature hormone. Activins stimulate *Fshb* expression via complexes of SMAD3, SMAD4, and FOXL2, which bind to the *Fshb* promoter. Recent evidence suggested additional regulatory mechanisms governed by an upstream enhancer. Addition of this enhancer increased activin-stimulated murine *Fshb* promoter-reporter activity in gonadotrope-like LβT2 cells. In addition, a variant in the corresponding human FSHB enhancer is associated with lower FSH levels. However, in contrast to the previous study, addition of a genomic region containing the enhancer did not increase basal or activin-regulated murine *Fshb* promoter-reporter activity in LβT2 cells. Moreover, FSH synthesis and secretion were unaltered in male or female mice in which the enhancer was deleted by CRISPR-Cas9. Single nucleus ATAC-seq analysis of wild-type murine pituitary demonstrated the presence of three additional regions of co-accessibility upstream of the *Fshb* gene exclusively in gonadotropes. We refer to these as enhancers (Enh) 1-4, with Enh 1 being most proximal to the gene. The previously described enhancer is Enh 2. The *Fshb* promoter and three out of four enhancers were fully or partly closed in gonadotropes of activin type II receptor knockout mice. According to ChIP-seq analysis in LβT2 cells, SMAD3, FOXL2, and the enhancer mark H3K27Ac were enriched in Enh 3 and 4. Unlike Enh 2, Enh 3 and 4 increased basal and activin-stimulated murine *Fshb* promoter-reporter activity in LβT2 cells. Enh 1 had similar but less robust effects. Collectively, the data indicate that there are as many as four upstream enhancers that regulate murine *Fshb* transcription. At least two of these regions bind SMADs and FOXL2, which may facilitate their association with the promoter.

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Benchmark Concentration Modelling and ToxPi Analyses of the Effects of Organophosphate Esters on Human Adreno-carcinoma (H295R) Cells

Zixuan Li, Bernard Robaire, Barbara F. Hales

The organophosphate esters (OPEs) that are used extensively as flame retardants and plasticizers are found ubiquitously in the environment. A few in vivo studies showed that exposure to some OPEs have detrimental effects on the adrenal gland. The goal of this study was to assess the effects of exposure to OPEs on the phenotype of adrenal cells. We hypothesized that OPEs alter the phenotype of adrenal cells to a greater extent than the legacy polybrominated diphenyl ether (PBDE) flame retardants that they have replaced. H295R adrenal cells were exposed (0.001-100μM) for 48h to commonly used OPEs: triphenyl phosphate (TPHP); tris(methylphenyl) phosphate (TMPP); tris(2-butoxyethyl) phosphate (TBOEP); tris(1,3- dichloro-2-propyl) phosphate (TDCIPP); or tris(1-chloro-2-propyl) phosphate (TCIPP)] or to a PBDE [2,2',4,4' tetrabromodiphenyl ether (BDE-47). High content screening was used to assess effects on cytotoxicity, oxidative stress, total and active mitochondria, lysosome intensity and numbers, and the total area of lipid droplets. Benchmark concentration (BMC) and toxicological prioritization index (ToxPi) analyses were used to rank these chemicals based on their potencies. The rank order of the cytotoxicity of the chemicals tested was: TMPP > TPHP > TDCIPP=BDE-47>TBOEP>TCIPP. Oxidative stress levels decreased after exposure to TPHP (BMC10: 22.9μM), TMPP (TMPP BMC10: 122.4μM), or TDCIPP (BMC10: 6.7μM). Although BDE-47 did not affect the total area of lipid droplets, each of the OPEs increased lipid droplet areas. TMPP was the most potent (BMC10: 0.4μM) and TCIPP the least (BMC10: 58.7μM). ToxPi rankings based on the overall bioactivities were TMPP>TPHP>TDCIPP>BDE-47>TBOEP>TCIPP. Non-triaryl OPEs (TBOEP, TDCIPP, and TCIPP) had higher BMC values and were ranked lower in ToxPi analyses than triaryl OPEs (TPHP and TMPP) with respect to most of the phenotypic endpoints tested, suggesting that they may be safer replacements for PBDEs than triaryl OPEs.

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Hanan Mohammad

The signaling signatures of biased D1 receptor agonists in vivo

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

G protein-coupled receptors (GPCRs) comprise the largest receptor superfamily encoded in the human genome and include many therapeutic drug targets. An individual GPCR can initiate several models of signaling inside the cell; some of these are therapeutically beneficial, with others result in the development of undesired side effects. "Biased" ligands are defined by their ability to preferentially promote or inhibit a subset of signaling cascades downstream of a given GPCR. Recently, a novel series of non-catechol biased D1 receptor (D1R) agonists was developed and showed limited tendency to recruit β-arrestin (i.e., G protein-biased). However, it is unclear to what extent the therapeutic and adverse effects of D1R agonists are driven by G protein vs. β-arrestin mediated signaling. In addition, biased signaling has been extensively studied in vitro, but little is known about how biased agonists perform in the more complex environment of the living brain. To this end, our labs have developed a novel technique based on Förster resonance energy transfer (FRET) to enable in vivo recording of GPCR signaling in awake and behaving rats. Our global goal is to utilize our in vivo biosensor approach to quantify the pharmacology of biased agonists by directly measuring signaling pathway engagement in healthy and diseased animals. We will express FRET-based biosensors by stereotaxic injection of adeno-associated viruses expressing biosensors into the brain region of interest. In vivo fiber-photometric recording will be performed using a chronically implanted fiber-optic probe at the site of biosensor expression. A broad panel of biosensors will be used to examine differential G-protein coupling, β-arrestin recruitment, and downstream signaling. Biased D1R signaling will be studied in healthy and dopamine lesioned rats. The project will provide a deeper understanding of how GPCR-dependent signaling contributes to the therapeutic and adverse effects of novel drugs.

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Mistrafficking of KCC2 in the Endolysosomal System in Christianson Syndrome Animal Model

Jamie Mustian, Andy Gao PhD, Roy Shi, Hannah Lindsay, and Anne McKinney PhD

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

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

Ali Shariat-Panahi, Jason C. Tanny

Regulators of transcription elongation by RNA Polymerase II (Pol II) can be targeted for the treatment of diseases such as cancer, making it crucial to understand their mechanism of action. Spt5 is a component of the Pol II elongation complex (EC) which directly binds the Pol II subunit, Rpb1. Both Rpb1 and Spt5 have unstructured C-terminal domains (CTDs) composed of short amino acid repeats. The Rpb1-CTD participates in the formation of molecular clusters during transcription through Liquid-Liquid Phase Separation (LLPS). LLPS properties of the Rpb1-CTD, as well as its interactions with other regulators, are controlled by the phosphorylation state of Rpb1-CTD. The Spt5-CTD is also phosphorylated during transcription, and genetic evidence suggests it plays vital roles in elongation. Spt5-CTD is also the site of one known phospho-dependent interaction with the elongation factor Rtf1. However, in contrast to the Rpb1-CTD, little is known about its function. We propose that the Spt5-CTD has analogous functions to the Rpb1-CTD in regulating transcription, including the formation of liquid-like droplets through LLPS and mediating the recruitment of EC components in a phosphorylation-dependent fashion. Herein, I use the fission yeast (S. pombe) for a detailed analysis of Spt5-CTD function. First, I will analyze the role of the known Spt5-Rtf1 interaction in Pol II elongation and termination. Secondly, proteomics analyses will be carried out to gain a more comprehensive understanding of Spt5-CTD interactions with other regulators. Finally, LLPS properties of the Spt5-CTD will be examined using recombinantly expressed Spt5-CTD constructs with varying repeat lengths. I expect that the outcomes of these experiments will help in deciphering the exact role played by the Spt5-CTD in transcription elongation/termination, which could provide novel avenues for treating human diseases.

Aknowledgements | They go here

AhR regulates pulmonary inflammation from cannabis smoke

Emily Wilson, David Eidelman, Carolyn Baglole

There is inconclusive literature on the effects of cannabis smoke (CaS) on lung inflammation. While Δ9-Tetrahydrocannabinol (THC) classically interacts with CB1 and CB2 receptors, non-G protein coupled receptors (GPCRs) can also interact with cannabinoids. This may include the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor highly expressed in the lungs that controls inflammation. We hypothesize AhR protects against the effects of CaS in the lungs. Age-matched (8-12 weeks) heterozygous (Ahr+/−) and knock-out (Ahr−/−) mice were exposed to two cannabis joints containing 0.5g dried cannabis using SCIREQ® inExpose system twice per day for 3 days. Plasma, bronchoalveolar lavage, and lung tissue were collected. THC ELISA was performed on the plasma. Cytokine profiling microarray was performed on the plasma and bronchoalveolar lavage. Immune cells of the lungs were assessed by flow cytometry. Inflammatory markers were analyzed by LC/MS. Expression of cannabinoid metabolizing enzymes was measured by qPCR. CaS activated the AhR only in Ahr+/- mice as indicated by increased CYP1A1 mRNA expression in the lungs. Neutrophils and inflammatory monocytes were higher in the lungs of Ahr-/- mice exposed to CaS. T-cells decreased in Ahr-/- mice. Plasma cytokines for neutrophil recruitment increased in Ahr-/- mice exposed to CaS while those for T-cells decreased. Cytokine expression in the airway of the lungs showed increases of eotaxin, LIF, VEGF, and IL-6 in both Ahr+/- and Ahr-/- mice treated with CaS, while the cytokines IP-10 and KC increased only in Ahr-/- mice exposed to CaS. Cannabinoid metabolizing enzyme expression in the lungs was induced by CaS in Ahr-/- mice only. Acute CaS exposure activates the AhR and increases inflammation in the absence of AhR expression. Overall, this work investigates the consequences of CaS on lung inflammation and its therapeutic potential in lung health, pointing toward a pivotal role for the AhR.

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

Dongwei (Oscar) Yu, Barbara F. Hales, Bernard Robaire

Organophosphate esters (OPEs) are used as flame retardants and plasticizers. Ingestion and inhalation of house dust is a major source of exposure. Three of the major OPEs detected in Canadian house dust are tris(2-butoxyethyl) phosphate (TBOEP), tri(methylphenyl) phosphate (TMPP), and triphenyl phosphate (TPHP). Here, we tested the hypothesis that these OPEs affect the liver; we used a high content imaging approach followed by benchmark concentration (BMC) modeling and toxicological prioritization index (ToxPi) analysis to compare their potencies in HepG2 cells. HepG2 cells were exposed for 48hr to vehicle control (DMSO) or each OPE at 0.001-100μM. Cells were screened using fluorescent dyes to elucidate effects on cytotoxicity, oxidative stress, mitochondria, lipid droplets and lysosomes. TBOEP was not cytotoxic to HepG2 cells; TMPP (IC50: 34.7μM; BMC¬10: 15.9μM) was more cytotoxic than TPHP (IC50: 97.8μM; BMC10: 31.5μM). Exposure to TBOEP (0.01-20μM), TMPP (0.001μM) or TPHP (0.1-20μM) reduced the fluorescent labelling for reactive oxygen species. TMPP and TPHP (0.001-50μM) significantly increased total mitochondria. TPHP (5-10μM) increased the staining for active mitochondria (BMC¬10: 42.2μM). Both TMPP (5μM; BMC¬10: 22.3μM) and TPHP (50μM; BMC10: 10.4μM) increased the size of lipid droplets. TBOEP (1-100μM), TMPP (5-10μM; BMC¬10: 2.8μM) and TPHP (20-50μM; BMC¬10: 4.8μM) all significantly increased the labelling of lysosomes. Thus, individual house dust OPEs have differential effects on liver cell phenotypic parameters. Using ToxPi analyses to integrate the effects on all phenotypic endpoints, the relative risk for these OPEs was: TPHP > TMPP > TBOEP.

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Garvit Bhatt

Structural and biophysical characterization of YopJ acetyltransferases

Garvit Bhatt, Jean-François Trempe, Bhushan Nagar

Bacterial pathogens block key intracellular pathways in their hosts to promote infection. Successful pathogens achieve this goal by injecting bacterial effector proteins directly into the host to turn off signaling proteins that activate pathways which promote inflammation and cell death. One such class of bacterial effectors are known as the YopJ acetyltransferases, which invade both plants and animals. Crystal structures of plant pathogen effectors have been determined, but structural insight on animal pathogen effectors remain a mystery and are important to understand structurally as the host targets of different bacterial effectors vary dramatically. My research aims to determine the structure of animal pathogen bacterial effectors in complex with the target host proteins to understand the molecular determinants that underlie this paradigm of the host-pathogen interface. This information will be used to develop chemical probes and ideally therapeutics that can modulate the interaction between pathogen and host, and hopefully treat diseases caused by autoimmune disorders and infections caused by Salmonella, Vibrio and Yersinia pestis.

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Seline Vancolen

Androgens upregulate pathogen-induced placental innate immune response

Seline Vancolen, Taghreed Ayash, Mariela Segura, Marie-Julie Allard*, Bernard Robaire*, Guillaume Sébire* (* Co-last authors)

Group B Streptococcus (GBS) is a leading cause of placental infection, termed chorioam-nionitis. Chorioamnionitis is associated with an increased risk of neurobehavioral impairments, such as autism spectrum disorders, which are more prominent in males than females. In a pre-clinical model of chorioamnionitis, a greater inflammatory response was observed in placenta associated with male rather than female fetuses, correlating with the severity of subsequent neurobehavioral impairments. The reason for this sex difference is not understood. Our hypothesis is that androgens upregulate the placental innate immune response in male fetuses. Lewis dams were injected daily from gestational day (G) 18 to 21 with corn oil (vehicle) or an androgen receptor antagonist (flutamide). On G 19, dams were injected with saline (control) or GBS. Maternal, fetal sera and placentas were collected for protein assays and in-situ analyses. Our results showed that while flutamide alone had no effect, a decrease in placental concentration of pro-inflammatory cytokines and infiltration of polymorphonuclear cells was observed in flutamide/infected compared to vehicle/infected groups. These results show that androgens upregulate the placental innate immune response and thus may contribute to the skewed sex ratio towards males observed in several developmental impairments resulting from perinatal infection/inflammation.

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Peiyou Wu

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

Pei You Wu, Luisa Speranza, Yanis Inglebert, Anna Francesconi, R. Anne McKinney

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

mGluR-LTD is critically involved in learning and memory formation in brain. Enhanced mGluR-LTD is associated with Fragile X syndrome, where an elevated level of SP is also observed. To understand the role of SP in mGluR-LTD, we used electrophysiology and imaging techniques and found that both functional and structural plasticity of hippocampal mGluR-LTD are impaired in SPKO. Furthermore, we found that although both mGluR1/5 activity contribute to mGluR-LTD, only mGluR5 activity is impaired in SPKO mGluR-LTD. Given that SP crosslinks with mGluR1/5 and their scaffolding protein, impaired mGluR5 activity could be due to reduced or mislocalized receptors. Using immunohistochemistry, we report that mGluR1/5 level and localization are not significantly changed in SPKO compared to WT. The findings suggest that the signaling pathways downstream of mGluR1/5 are disrupted in SPKO.

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Heme metabolites contribute to pathological angiogenesis in retinopathy of prematurity

Tapan Agnihotri, Nicholas Kim, Gael Cagnone, Jin Sung Kim, Emilie Heckel, José Carlos Rivera, Charlotte Bentus, Sheetal Pundir, Perrine Gaub, Florian Wunnemann, Walter Szarek, Hyman Schipper, Gregor U. Andelfinger, Kostas Pantopoulos, Jean-Sebastien Joyal

Retinopathy of prematurity (ROP) is a retinal disease in immature newborns characterized by pathological neovascularization. ROP classically arises due to changes in oxygen availability. Other factors, such as red blood cell (RBC) transfusion, independently contribute to disease severity. Heme molecules, abundant in RBC, are primary source of endogenous iron. Heme is metabolized by heme oxygenase (Hmox) into biliverdin, carbon monoxide (CO), and ferrous ions. Intracellular iron is an essential co-factor regulating HIF-1α degradation and is also known to be toxic in its free state. Also, CO is thought to be proangiogenic. Hence, both iron and CO could play a role in pathological angiogenesis. Microglia, have been shown to scavenge the microenvironment post hemorrhage and Hmox1 is one of the most highly transcribed genes in these cells in murine model of ROP. We, therefore, hypothesize that Hmox1 plays a critical role, both in pathological neo-vessel formation and in microglial scavenging response to tissue damage in ROP.

Single-cell RNAseq confirmed that Hmox1 and Vegfa were predominantly produced by retinal microglia and Müller cells respectively in oxygen induced retinopathy (OIR). Hmox1 expression was selective to pathological neo-vessels. Hmox1-deletion in microglial cells increased vaso-obliteration and its allosteric inhibition decreased pathological NV in OIR exposed mice. Further, microglia were seen to co-localize with the pathological neovessels in OIR exposed mice. In vitro, hemin and hypoxia increased Hmox1 protein expression in human microglial cells. Furthermore conditioned media from hypoxia and hemin-treated microglial cells displayed an increase in Vegfa expression in Müller cells. CO decreased mitochondrial respiration and ATP production in Muller cells, also resulting in VEGFA expression. Hence, our preliminary results suggest that heme metabolism by Hmox1-expressing microglial cells might help regenerate the damaged neurovascular retina in OIR.

Assessing brain nicotinic receptor function in vivo during chronic nicotine exposure and withdrawal

Lucas Marques, Paul Clarke

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

In this project, which has required much troubleshooting, three brain areas are targeted with AAV-delivered biosensors: the nucleus accumbens shell (dopamine biosensor GRABDA), and the ventral tegmental area (VTA) and medial geniculate nucleus (calcium indicator GCaMP6). The effects of chronic nicotine and subsequent withdrawal on calcium-related neuronal activity will be determined in the VTA and MGN. In the accumbens shell the effect of chronic nicotine and subsequent withdrawal on dopamine dynamics will be analyzed. It is hypothesized that chronic nicotine administration will reduce neuronal calcium and dopamine responses to acute in vivo nicotine challenge, indicating nAChR desensitization, and that during withdrawal the same neurons will produce an exaggerated response to an acute nicotine challenge, indicating nAChR supersensitivity.

This project will potentially provide the first in vivo evidence of supersensitive neuronal responses in nicotine withdrawal. Such findings would seriously question the rationale underlying nicotine replacement therapy and force a reconsideration in how drugs are used to treat nicotine addiction.

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Louis-Charles Masson

Downregulation of glutamate transporter EAAT1 as a result of endosomal mis-trafficking is involved in Christianson Syndrome ataxia

Louis-Charles Masson, Tsz Chui Sophia Leung, Jack Legler, Alanna J. Watt, R. Anne McKinney

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

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Xiaotong (Vicky) Wang

Benchmark concentration (BMC) modeling and ToxPi analyses for potency ranking of organophosphate Esters (OPEs) in KGN human granulosa cells

Xiaotong Wang, Barbara F. Hales, Bernard Robaire

Organophosphate esters (OPEs), used extensively as flame retardants and plasticizers, are ubiquitous in the environment. However, little information is available on their safety. The goal of this study was to elucidate the effects of OPEs on the phenotype of KGN human granulosa cells. We hypothesized that OPEs alter the phenotype of these cells to a greater extent than the legacy polybrominated diphenyl ether (PBDE) flame retardants that they have replaced. KGN cells were exposed (0.001–100μM) to an OPE [triphenyl phosphate (TPHP); tris(methylphenyl) phosphate (TMPP); isopropylated triphenyl phosphate (IPPP); tert-butylphenyl diphenyl phosphate (BPDP); tris(2,4-di-tert-butylphenyl) phosphate (TDtBPP); 2,4-bis(1,1-dimethylethyl)-phenol phosphate (BDMPP); tributoxyethyl phosphate (TBOEP); tris(1,3-dichloro-2-propyl) phosphate (TDCIPP); tris(1-chloro-2-propyl) phosphate (TCIPP)] or a PBDE [2,2',4,4' tetrabromodiphenyl ether (BDE-47)] for 48h. Effects on cell counts, cell viability, oxidative stress, total and active mitochondria, lysosome counts, Lysotracker Red intensity, and total lipid droplet areas were determined using high-content imaging. The test compounds were ranked based on their lowest concentrations at which a 10% response was observed; the rank order was: TMPP > IPPP > TDCIPP > BDE-47 > TPHP > BPDP > BDMPP > TBOEP > TCIPP = TDtBPP. The lowest observed BMC, was for the effect of TMPP on lipid droplets (BMC10: 1.2 μM). A complementary analysis using the ToxPi tool ranked the chemicals based on their overall bioactivities; the rank order was: TMPP > BPDP > IPPP > BDE-47 > TPHP > TDCIPP > BDMPP > TBOEP > TCIPP = TDtBPP. Herein, we identified some OPEs that are consistently ranked higher than BDE-47, and some OPEs that affected fewer parameters, suggesting that they may have potentially lower hazards. We propose that these approaches serve to identify chemicals most likely to be responsible alternatives.

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Kyla Bourque

A tale of three cell types: moving beyond HEK 293 cells towards primary and iPSC-derived cardiomyocytes to understand disease-relevant signalling

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The biology of GPCRs has frequently been studied in immortalized cell systems such as the HEK 293 cells. As these cells express several GPCRs and their G protein partners, they have provided valuable information regarding receptor function. To complement studies performed in HEK 293 cells, primary in vitro cell cultures from animal models have also been used, however, species differences may limit translatability of results obtained to human disease and its management. This is an important consideration as several studies have indicated that receptor function depends greatly on the cellular context as expression of GPCRs and effectors is cell-, tissue-, and could be patient specific. To dissect the importance of cell context, we examined expression data and signalling signatures in three cell types: HEK 293 cells, primary rat neonatal cardiomyocytes (RNCMs) and human iPSC-derived cardiomyocytes. Differential gene expression patterns were observed suggesting that GPCRs, heterotrimeric G proteins as well as their associated effectors are cell type specific. This supports the need to measure receptor function in a relevant physiological or pathophysiological context especially when interested in understanding how receptor function evolves during disease progression. Moreover, we explored intracellular signalling of endogenous receptors in RNCMs and iPSC-CMs using a number of biosensors that assessed ERK1/2 and PKA activity in the cytosol as well as in the nucleus. Over a 60-minute time period, differences were observed in the response to ligands that bind α- and β-adrenergic receptors. We also assessed signalling profiles in iPSC-CMs derived from patients diagnosed with dilated cardiomyopathy. Preliminary data suggests that signalling responses may be patient- and sex-specific. We anticipate that signalling profiles obtained will be informative and will aid to identify tailored therapeutic approaches to improve outcomes in cardiovascular disease.

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Age related gene expression changes in round spermatids of Brown Norway rats

Heather E Fice, Bernard Robaire

There is growing awareness of the effects of aging on the reproductive health of men and the consequences for their offspring. Correlative epidemiological studies examining paternal age and offspring health suggest there are more frequent occurrences of genetic disorders in the children of older men. As these are commonly multigene disorders, we hypothesize that their source is a disruption of the sperm epigenome and subsequently gene expression. We aim to characterize gene expression changes with aging, with focus on previously established processes that are disrupted with advanced paternal age. Round spermatids (RS) were collected from young (mean=5.3 months) and aged (mean=19.5 months) Brown Norway rats, representative of humans aged 20-30 years and 55+ years, respectively. mRNA sequencing (n=5) was done to examine gene expression through paired-end sequencing. Gene expression data were analysed for differential expression considering genes with a greater than 1 log2-fold change. Sequencing data display 221 upregulated and 9 downregulated transcripts in RS of aged rats, compared to young (log2FC >1, p <0.05). The dysregulated transcripts are involved in processes that are known to be disrupted with advanced paternal age, such as genes that are involved in sperm-egg binding, oxidative stress response, cell motility and morphology. There is increased expression of the folate acid receptor transcripts Folr1 (log2FC 4.06) and Folr2 (log2FC 3.70) This lends a potential source to the alternation observed in DNA methylation levels with aging, as folate is used as a methyl donor. These results show an overall increase in RS gene expression with age, with spermatogenic functions being perturbed. Taken together, these findings suggest genetic origins of the fertility, and DNA packaging effects observed with advanced paternal aging.

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Sarah MacKinnon

The CTD of transcription elongation factor Spt5 regulates heterochromatin through dual mechanisms

Sarah MacKinnon, Viviane Pagé, Ryan Martin, Stewart Shuman, Beate Schwer, Jason C. Tanny

Silencing of repetitive DNA in eukaryotic genomes is important for maintenance of genomic stability. This silencing operates at the transcriptional level through formation of compacted chromatin structures termed heterochromatin, characterized by conserved histone modifications such as methylation of histone 3 on lysine 9 (H3K9me). In the model Schizosaccharomyces pombe (S. pombe), heterochromatin is linked to the RNAi pathway, with siRNAs providing sequence specificity to an Argonaute family protein, allowing it to target H3K9me to complementary loci. The RNAi-dependent heterochromatin pathway is the major pathway that results in pericentromeric heterochromatin nucleation, although a less understood RNAi-independent pathway also exists. Paradoxically, siRNA-driven heterochromatin formation requires transcription. Consequently, transcriptional regulators can have important roles in forming heterochromatin. During transcription, the C-terminal domain (CTD) of the elongation factor Spt5 is phosphorylated by Cdk9, allowing it to promote co-transcriptional histone modifications. The CTD is composed of 18 repeats of a nonapeptide consensus sequence. We hypothesized that mutations in the Spt5 CTD would result in dysregulated heterochromatin formation.

We tested an array of Spt5 CTD mutants that differ in repeat length and phosphorylation status in order to differentiate phosphorylation- and length-dependent effects of the Spt5 CTD in regulating heterochromatin formation. Using reporter gene assays and complementary H3K9me-specific ChIP assays, we found that mutations that disrupt phosphorylation of the Spt5 CTD result in ectopic heterochromatin formation and spreading, phenotypes linked with increased RNAi activity. Unexpectedly, truncating the CTD was able to rescue heterochromatin levels in the absence of the RNAi pathway. These results suggest that the Spt5 CTD is able to regulate heterochromatin levels through both the RNAi- dependent and -independent pathways.

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Anne-Sophie Pépin

Paternal diet-induced obesity alters the sperm epigenome and the placental transcriptome in a sex-specific manner

Anne-Sophie Pépin, Vanessa Dumeaux, Deborah M. Sloboda, and Sarah Kimmins

Growing evidence point towards a strong contribution of paternal factors in placental health with implications in adult-onset complex disease risk. We have recently demonstrated that paternal diet-induced obesity alters sperm histone methylation and is associated with metabolic disturbances in the next generation. Diet-sensitive epigenetic regions in sperm were found at genes involved in trophectoderm and placental development, and corresponded to epigenetic and gene expression profiles in these tissues. We sought to investigate whether paternal diet-induced obesity before conception can alter the placental transcriptome, and whether differential gene expression corresponds with sperm obesity-associated epigenetic signatures. C57BL6/J males were fed either a control or high-fat diet for 10 weeks beginning at 6 weeks of age. They were then bred to control-fed C57BL6/J females to induce pregnancies and E14.5 placentas were collected. RNA-sequencing was performed (n=4 per group per sex) to detect sex-specific transcriptional changes associated with paternal diet. At necropsy, sperm was collected for chromatin immunoprecipitation followed by sequencing (ChIP-seq; n=3 per group) targeting histone H3 lysine 4 tri-methylation (H3K4me3) to detect obesity-induced changes in H3K4me3 enrichment. There were sex-specific differentially expressed genes in placentas with some overlapping with promoters showing obesity-associated sperm epimutations. Using single-cell RNA-seq data from mouse E14.5 placenta (Han et al., Cell, 2018), we deconvolved the bulk RNA-seq data to assess whether paternal diet affects placental cell type proportions and whether there are cell-type-specific signatures associated with paternal diet. This study highlights a previously underappreciated role of the placenta at the origin of paternally-induced metabolic disturbances in offspring.

GD2 and GD3 tumor gangliosides are diagnostic markers for all subtypes & stages of epithelial ovarian cancer

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Ovarian cancer (OC) is the deadliest gynecological cancer with low cure rates that have remained unchanged for three decades. A major contributor to this poor survival is the lack of early diagnostic tools that result in true disease identification at late cancer stages. Seeking to alleviate this unmet diagnostic need, we evaluated two cancer biomarkers, gangliosides GD2 and GD3 glycolipids, not previously investigated for diagnostic purposes in OC. We show by immunohistochemistry (IHC) on ovarian tumor microarrays that GD2 and GD3 are detected uniformly in 78% of all OC subtypes and at all stages of the disease (n= tissues from 214 patients). In controls, GD2 and GD3 were not present in surrounding healthy tissues, or in ovarian tissue from non-OC patients. Since GD2 and GD3 are shed by tumor cells, we developed a quantitative ELISA method measuring GD2 and GD3 in serum. The ELISA was used for a discovery study (n=379 serum samples) and a powered retrospective study (n= 200 serum samples) for building a preliminary but cross validated diagnostic model. Individually the GD2 and GD3 serum tests achieved significant OC detection, including the hard-to-diagnose early-stage I/II, and the low-CA125 OC cohort. Combining GD2+GD3+age (AUC 0.983 overall and 0.969 for early-stage) is significantly superior to the standard of care CA125 (AUC 0.855 overall and 0.700 for early-stage).

Prospective clinical trials could yield an effective liquid biopsy diagnostic for early-stage ovarian cancer, to improve overall survival.

Gold nanoclusters modulate the state of human neural cells

Maja Beus, Sabina Kerbalaeva, Evan Rizzel Gran, Željka Sanader Maršić, Dusica Maysinger

Rationale: Gold nanoclusters (AuNCs) are atomically precise gold nanostructures with unique photophysical properties. Their properties depend on the size, spatial Au atom arrangement and attached ligands. However, there is limited information available about AuNCs' behavior in neural cells.

The aim of this study was to show the relationship between some physical and biological properties of several AuNCs focusing on those with 25 Au atoms and two small biocompatible ligands (N-acetylcysteine and glutathione) in human microglia (HMC3), neuronal cells (differentiated SH-SY5Y) and primary human astrocytes.

Experimental approaches and results: Cell counting showed no change in cell numbers relative to the control at concentrations <10 μM. AuNC with 25 Au atoms and 18 N-acetylcysteine residues (Au25AcCys18) reduced abundance of reactive oxygen species (ROS) as determined by CellRox fluorescence in both HMC3 and differentiated SH-SY5Y cells, but did not do it in human astrocytes. Au25AcCys18 decreased L-buthionine sulfoximine (BSO)-induced oxidative stress. Acetylated HMGB1 was more abundant in neuron-like cells than in human microglia suggesting their greater responsiveness to Au25AcCys18. Minimum number of cells analysed per condition was 67. Statistical analysis: data are expressed as mean±SEM. Statistical comparisons between groups were conducted by unpaired Student's t-test. *indicates $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$. The value of $p < 0.05$ was considered statistically significant.

Conclusions: Au25AcCys18 exert cell-type dependent effects. They are not cytotoxic in concentrations < 10 μM, and they reduce ROS abundance under oxidative stress conditions. Future direction: Luminescent properties of gold nanoclusters can be exploited for label-free bioimaging and combination therapy in inflammatory disorders and cancer chemotherapy.

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The effects of bisphenol A and its structural analogs on the expression of genes involved in the regulation of steroidogenesis in MA-10 mouse tumour Leydig cells

Lama Iskandarani, Sabrina Romanelli, Bernard Robaire, Barbara F Hales

Bisphenol A (BPA) is the main monomer of polycarbonate plastics and epoxy resins, and is widely used in consumer products such as reusable plastic water bottles. BPA is an endocrine disrupting chemical that is associated with a variety of adverse effects in humans and in animal models; thus, many alternatives to BPA are now emerging in consumer products, such as BPAF, BPF, BPS, BPM, and BPTMC. Exposure to BPA is linked to alterations in testicular steroidogenesis, leading to changes in steroid hormone levels. However, little is known about the impact of structural analogs of BPA and the mechanism(s) by which they may affect the steroidogenic pathway. We showed previously that BPA and five of its analogs increase the levels of progesterone produced by MA-10 cells. To determine the effects of these chemicals on the key enzymes involved in the steroidogenic pathway, we cultured MA-10 cells in the presence of vehicle, BPA, BPAF, BPF, BPS, BPM, and BPTMC under both basal and dibutyryl-cAMP (Bu2cAMP) stimulated conditions. Star was stimulated by Bu2cAMP, leading to a drastic increase in its mRNA levels compared to basal levels. This expression was further increased after exposure to BPA and all of its analogs. The expression of Tspo, Cyp11a1, Hsd3b1, Hmgcr, and Srebf2 was not affected by Bu2cAMP. Exposure to BPA, BPAF, BPM, and BPTMC decreased the expression levels of Tspo and Cyp11a1 under both basal and stimulated conditions. Hsd3b1, Hmgcr, and Srebf2 were generally downregulated after exposure to all six bisphenols. Our data show that only Star expression is increased both by Bu2cAMP and exposure to bisphenols. This increase in Star may increase the import of cholesterol into the inner mitochondrial membrane and contribute to the increase in progesterone levels we observed. In conclusion, BPA's structural analogs all affect the expression levels of several gene transcripts; we suggest that they may not be responsible replacements for BPA in consumer products.

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Deciphering e-cigarette mediate effects on pulmonary innate immune cells

Amelia Kulle, Martin Mawhinney, Ajitha Thanabalasuriar

Smoking tobacco has caused a major burden on the public health system for many years and is the leading cause of preventable death in North America. The introduction of e-cigarettes was advertised as a healthy alternative to smoking cigarettes. Unfortunately, these products have gained popularity among the adolescent population. With the relative novelty of these devices, many of the health effects remain unknown. One major concern of e-cigarette use includes the outbreak of e-cigarette or vaping associated lung injury (EVALI) among adolescents, marked by the sudden infiltration of leukocytes. Scientific evidence is lacking in understanding the mechanism by which the use of these devices impacts different systems in our body. For this project we seek to understand how innate immune cell infiltration is affected by different components in e-cigarette liquid (e-liquid). Our lungs have a resident population of immune cells that are critical for maintaining homeostasis. However, when their activation is dysregulated, these cells can cause excess inflammation leading to airway damage. By understanding the mechanisms by which different components of e-liquid disrupt the balance of the pulmonary immune environment leading to conditions like EVALI, we can then advise public health policies for harm reduction.

Myostatin is the primary TGFβ ligand driving follicle-stimulating hormone synthesis in mice

Luisina Ongaro, Xiang Zhou, Ying Wang, Gauthier Schang, Emilie Brule, Yeu-Farn Lin, Roselyne Castonguay, Ulrich Boehm, Gloria Su, Se-Jin Lee, and Daniel J. Bernard

Activins were discovered based on their abilities to stimulate follicle-stimulating hormone (FSH) secretion by the pituitary gland. According to current dogma, autocrine/paracrine activin B produced by pituitary gonadotropes stimulates transcription of the FSHβ (*Fshb*) subunit gene. Consistent with this idea, mice lacking activin type II receptors (ACVR2A and ACVR2B) in gonadotropes are FSH deficient and infertile. In contrast, however, activin B deficient (*Inhbb* knockout) mice have elevated FSH levels. Therefore, either activin B does not regulate FSH synthesis or another related TGFβ ligand can compensate in its absence. Conditional ablation of the activin type I receptor, ACVR1B, in gonadotropes did not affect FSH production, ruling out a role for activin A. In contrast, gonadotrope-specific deletion of the TGFβ type I receptor, TGFBR1, led to reduced FSH and subfertility. Mice lacking both ACVR1B and TGFBR1 in their gonadotropes were FSH deficient and sterile. Myostatin and GDF11 are TGFβ family members that signal via ACVR2A/ACVR2B- and TGFBR1/ACVR1B. To assess their potential roles in FSH regulation, we treated wild-type mice with the bioneutralizing antibodies RK-22 (myostatin-specific) and RK-35 (myostatin and GDF11). Both antibodies rapidly and significantly decreased FSH production as well as ovulation in females. Moreover, FSH levels were significantly reduced in male global *Mstn* knockout relative to wild-type mice. Myostatin is predominantly expressed in skeletal muscle and FSH levels were also decreased in muscle-specific *Mstn* knockouts (*Myl1*-Cre) relative to controls. Collectively, the data indicate that myostatin is a major regulator of FSH synthesis in mice, both challenging existing dogma and revealing a novel muscle-pituitary gland endocrine axis.

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Ai Liu

Characterization of myelin deficits in rats with Alzheimer's disease

Ai Liu, Sonia Do Carmo,A. Claudio Cuello

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects daily living through memory loss and cognitive impairment. One hallmark of Alzheimer's disease is abnormal tau accumulation, forming tangles inside neurons which coincide with neurodegeneration and cognitive dysfunction. It is also reported that myelin damage is common in dementia and may contribute to functional decline. However, there is no direct evidence on the relationship between tau pathology and demyelination.

Method: To explore the effects of a progressive tauopathy on myelin, we will apply our newly generated McGill-R955-hTau transgenic rats, which express the longest form of human tau with the P301S mutation. To better correlate the effects of tauopathy-driven myelin defects with cognition, we have first characterized the cognitive function of McGill-R955-hTau heterozygotes and homozygotes transgenic rats at 12 months and 20 months in comparison to wild-type rats. For this we applied behavior tests such as open field, novel object, Y-maze, social interaction, Morris water maze and auditive fear conditioning.

Results: Homozygous McGill R955-hTau transgenic rats display cognitive impairment in specific behavior tests at 20 months of age which are more pronounced than in heterozygous rats. At 12 months of age, although tau hyperphosphorylation at Ser202/Thr305 is present, homozygous McGill R955-hTau transgenic rats do not show impairments in the tests applied.

Conclusion: Overall, these findings indicate that McGill-R955-hTau rats represent a viable model to examine mechanisms underlying age-related and tau gene-dose dependent effects on behavior and neuronal function in Alzheimer's disease. Our next step will be to examine region-specific alterations in myelination patterns and in oligodendrocyte marker expression profiles at time points preceding the appearance of advanced tau pathology.

Benjamin Kannel

Does inhibiting the amyloidogenic pathway in Down Syndrome ameliorate NGF metabolic dysfunction?: Implications for the induction of a major disease aggravating pathology in Alzheimer's Disease

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

In Alzheimers Disease, the atrophy and degeneration of the cholinergic neurotransmitter system, beginning with the basal forebrain cholinergic system, is central to the onset of symptoms. This basal forebrain cholinergic system relies solely on mature nerve growth factor (mNGF) for it's trophic support. In this presentation I put forth evidence for a failed metabolic pathway of NGF in the AD continuum, beginning at preclinical stages and correlating to cognitive loss as well as loss of cholinergic tone. As Down Syndrome (DS) individuals are on a predetermined track for Alzheimers Disease (due to the triplication of the APP gene on the 21st chromosome), and DS foetal primary cortical cell cultures display concommittent amyloidoises and NGF dysmetabolism, and are an accessible and manipulatable sample set, they are therefore ideal for my current work in investigating if amyloid beta may be the mechanism of induction for this NGF dysmetabolism. Further, I detail where I am at in this work, and the expected outcomes and implications of this work.


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