PROGRAMME CONFÉRENCE / CONFERENCE PROGRAM

Canadian Oxidative Stress Consortium

11th Meeting of the Canadian Oxidative Stress Consortium (COSC 2023)
Montreal, Quebec, Canada
May 10-12, 2023

Website: https://event.fourwaves.com/cosc2023

Conference location: Jewish General Hospital, **Block Amphitheatre B-106**
3755 chemin de la Côte-Ste-Catherine, Montreal, QC H3T 1E2

Jewish General Hospital
Lady Davis Institute for Medical Research

Rev 4_May 11, 2023

Canadian Oxidative Stress Consortium

Conférenciers de prestige / Keynote Speakers

Dr Helmut Sies, Institute of Biochemistry and Molecular Biology, Heinrich-Heine University Düsseldorf, Germany
Dr Marcus Conrad, Institute of Developmental Genetics, Helmholtz Zentrum München, Germany
Sharon Campbell, Lineberger Cancer Center, University of North Carolina, Chapel Hill, USA

Comité organisateur local / Montreal Local Organizing Committee

Diana Averill-Bates, Co-chair, Université du Québec á Montréal
Kostas Pantopoulos, Co-chair, Lady Davis Institute, McGill University
Volker Blank, Lady Davis Institute, McGill University
Ann English, Concordia University
Ryan Mailloux, McGill University
Charles Ramassamy, INRS-Centre Armand-Frappier-Santé Biotechnologie (INRS-CAFSB)

Comité organisateur national / National Organizing Committee

William Willmore, COSC Treasurer, Carleton University
Chris Perry, York University
Scott Ryan, University of Guelph
Arno Siraki, University of Alberta
Jim Uniacke, University of Guelph

Secretariat:
Gabriella Di Pancrazio
cosc.oxistressmtl@gmail.com

Jewish General Hospital
Lady Davis Institute for Medical Research

All lectures will be held in Block Amphitheatre B-106, 1st Floor of the Jewish General Hospital 3755 chemin de la Côte-Ste-Catherine, Montreal, QC H3T 1E2

Dear Colleagues,

*On behalf of the organizing and scientific committee for the **11th Meeting of the Canadian Oxidative Stress Consortium (COSC 2023)**, welcome to Montreal. We believe that this spring conference, set in our beautiful cosmopolitan city, will reflect the best of oxidative stress research.*

The COSC research network advances research and education in oxidative stress in health and disease. COSC was initiated in July 1999, and during the past 24 years has grown to include researchers from over 60 laboratories in over 20 institutions across Canada. Oxidative stress (a key component of redox biology) is implicated in a broad range of health disorders including cancer, aging-related diseases, ischemia-reperfusion injury, stroke, diabetes, cardiovascular and respiratory diseases, arthritis, neurodegenerative diseases such as Alzheimer's and Parkinson's, spinal cord injury, antibiotic resistance, sepsis, and inflammation. Oxidative stress has relevance in fields such as plants, microorganisms, toxicology, and environmental sciences.

COSC is managed by a volunteer Executive Committee comprised of ten senior, mid-career, and junior professors from different Canadian universities. Our main scientific event is our meeting every two years on the campus of a Canadian university or research institution. COSC fosters research collaboration among its members, provides excellent training opportunities for students and postdoctoral fellows, enabling them to learn new techniques and access state-of-the-art technology across the Consortium, and promotes close ties with the pharmaceutical industry.

Notably, COSC actively seeks interactions and collaborations with new Canadian researchers in the field. Our junior faculty members are highly active, and several have hosted a COSC meeting at their home institution or plan to do so.

Training tomorrow's scientific leaders is a major focus and COSC meetings provide outstanding networking opportunities as well as lots of time for trainees to informally discuss their research with top experts in the field. Dedicated trainee symposia are scheduled at COSC 2023 to give graduate students and postdoctoral fellows ample opportunity to present their research, to hone their skills at effectively delivering oral and poster presentations, to network and to engage in scientific discussions. Furthermore, trainees are rewarded for exceptional participation; travel awards are provided based on abstract selection and monetary rewards are bestowed for outstanding oral and poster presentations.

The biennial COSC conference continues to attract new participants and we are particularly impressed by the breadth and caliber of the research to be presented in this year's program. One of the most exciting aspects of organizing our conference is that we can provide a time and place to showcase the work of investigators at all stages of their careers and promote networking as we have much to learn from one another. Such diversity makes our meeting the premier Canadian conference in the field of oxidative stress.

The last COSC meeting was held in Edmonton in 2018. The Montreal local organising committee (LOC) started organising the next meeting in 2019 as it was originally scheduled to be held in May 2020. However, we had to postpone the meeting in mid-March 2020 because of the lockdown caused by the Covid-19 pandemic. We postponed the meeting to May 2021, then to May 2022. Zoom meetings are not in the tradition of COSC as we value in-person interactions between colleagues, trainees, and experts in the field. So here we are in Spring 2023 and COSC 2023 will finally be held in person. The Montreal LOC has been organising this meeting for 4 years!

Events such as COSC 2023 require many minds and even more hands to be executed successfully. We continue our good fortune through the enormous local support within the four Montreal Universities and affiliated research institutes, as well as nationally and internationally.

Of particular note, COSC 2023 is supported by a generous conference grant from the Canadian Institutes of Health Research (CIHR), and sponsorship funding from the Society for Redox Biology and Medicine, the Society for Free Radical Research – Europe, the Lady Davis Institute for Medical Research, the Jewish General Hospital, McGill University Faculty of Medicine, the Fondation Armand Frappier, Université du Québec à Montreal, Concordia University; the three companies, Roche, Nikon and Wisent; and the two journals, Antioxidants and Biochimica Biophysica Acta - Molecular Cell Research.

To inspire imaginations and provide respite from PowerPoint and posters, we have arranged a very special gala dinner for this year's awards ceremony at the Bonaventure Hotel on the evening of Friday, May 12. On Tuesday evening, May 9, we will hold a Meet and Greet networking event from 7-9 pm at Hurley's Irish Pub, an authentic Irish landmark in the heart of downtown Montreal since 1993:
http://www.hurleysirishpub.com/en_home.html
Attendees at COSC 2023 are invited to submit an article to be published in a conference special issue in the journal Biochimica Biophysica Acta - Molecular Cell Research. The special issue will be entitled "Oxidative Stress in Health and Disease". The deadline for submission of articles for this special issue is November 30, 2023. BBA - Molecular Cell Research focuses on understanding the mechanisms of cellular processes at the molecular level and is one of the ten topical journals of the BBA series of international journals.
Your evenings will be free to visit and explore our beautiful city. For example, you can walk to historic Old Montreal and the Old Port as well as enjoy the buzz of the downtown core.

Thank you for joining us this year and let's get started!

Sincerely,

Diana Averill-Bates, PhD
COSC 2023 Co-Chair
Université du Québec à Montréal

Kostas Pantopoulos, PhD
COSC 2023 Co-Chair
Lady Davis Institute for Medical Research / Jewish General Hospital
McGill University

Montreal Local Organising Committee:
Ann English *(Concordia)*
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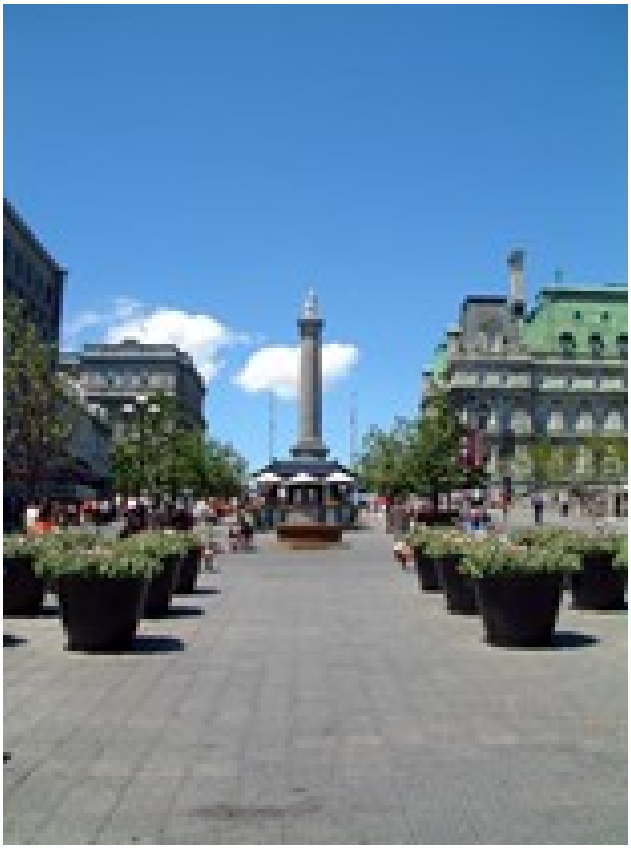


Chers collègues,

*Au nom du comité organisateur et scientifique de la **11è Rencontre du Consortium canadien sur le stress oxydatif (COSC 2023)**, nous vous souhaitons la bienvenue dans la superbe ville cosmopolite de Montréal au printemps. Nous sommes certains que cette conférence reflétera le meilleur de la recherche sur le stress oxydatif.*

COSC est un réseau de recherche dont l'objectif est faire progresser les connaissances, la recherche et l'éducation sur le stress oxydatif en santé et dans les maladies. Le COSC a démarré il y a 24 ans, en juillet 1999. Ce Consortium Canadien s'est développé au cours des dernières décennies et compte maintenant des chercheurs de plus de 60 laboratoires provenant de plus de 20 établissements à travers le Canada.

Le stress oxydatif (biologie du rédox) est impliqué dans un large éventail de troubles de la santé, notamment le cancer, les maladies liées au vieillissement, les lésions d'ischémie-reperfusion, les accidents vasculaires cérébraux, le diabète, les maladies cardiovasculaires et respiratoires, l'arthrite, les maladies neurodégénératives telles que la maladie d'Alzheimer et de Parkinson, les lésions de la moelle épinière, la résistance aux antibiotiques, la septicémie et l'inflammation. Le stress oxydatif joue aussi un rôle important dans des domaines tels que les plantes, les micro-organismes, la toxicologie et les sciences de l'environnement.

Le COSC est géré par un comité exécutif bénévole composé de dix professeurs seniors, à mi-carrière et juniors provenant de différentes universités canadiennes. L'événement scientifique principal du COSC est de tenir une réunion tous les deux ans sur le campus d'une université ou d'un institut de recherche. Le COSC encourage la collaboration en recherche entre ses membres, offre d'excellentes opportunités de formation aux étudiants et aux stagiaires postdoctoraux, leur permettant d'apprendre de nouvelles techniques et d'accéder à des technologies de pointe dans l'ensemble du Consortium, et favorise des liens étroits avec l'industrie pharmaceutique.

Notamment, le COSC est un réseau qui encourage des interactions et des collaborations actives avec de nouveaux chercheurs canadiens dans le domaine. Ces membres juniors du corps professoral sont très actifs et plusieurs d'entre eux ont organisé une réunion du COSC dans leur établissement d'origine ou prévoient de le faire.

La formation des leaders scientifiques de demain est un objectif majeur et les réunions du COSC qui offrent des opportunités de réseautage exceptionnelles aux stagiaires et de nombreuses occasions de discuter de manière informelle de leurs recherches avec les meilleurs experts du domaine. Dans ce cadre, des symposiums sont dédiés aux étudiants diplômés et aux boursiers postdoctoraux au COSC 2023 pour leur offrir l'opportunité de présenter leurs recherches, de perfectionner leurs compétences pour présenter efficacement des présentations orales et par affiches, de réseauter et de participer à des discussions scientifiques. De plus, des prix sont prévus pour récompenser leur participation exceptionnelle tels que des bourses de voyage sont attribuées sur la base de la qualité des résumés et des récompenses monétaires sont accordées pour des présentations orales et par affiches exceptionnelles.

Notre conférence bi-annuelle continue d'attirer de nouveaux participants et nous sommes fiers de la qualité du programme proposé cette année. L'un

des aspects les plus passionnants de l'organisation de cette conférence bi-annuelle est que nous pouvons offrir un lieu et un moment pour présenter les travaux de - et de réseauter pour - des chercheurs à toutes les étapes de leur carrière. Nous avons beaucoup à apprendre les uns les autres. Ce type de diversité fait de notre rencontre la première conférence canadienne dans le domaine du stress oxydatif.

La dernière réunion du COSC a eu lieu à Edmonton en 2018. Le comité organisateur local (COL) de Montréal a commencé à organiser la réunion bisannuelle du COSC 2020 en 2019 et elle devait initialement se tenir en mai 2020. Cependant, nous avons dû reporter la réunion moins de 2 mois avant l'événement en raison du confinement à Montréal à la mi-mars causé par la pandémie de Covid-19. Nous avons reporté la réunion en mai 2021, puis mai 2022, et ainsi de suite. Les réunions Zoom ne sont pas dans la tradition du COSC où nous valorisons les interactions en personne entre collègues, stagiaires et experts du domaine. Nous nous réjouissons enfin de la tenue en présentiel du COSC 2023 que le COL de Montréal organise depuis 4 ans !

Des événements comme celui-ci nécessitent le dévouement de nombreuses personnes pour être exécutés avec succès. Nous bénéficions d'un énorme soutien local des quatre universités montréalaises et des instituts de recherche affiliés, ainsi que ceux à l'échelle nationale et internationale.

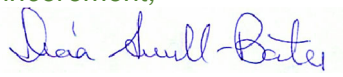
Il convient de noter en particulier que cette conférence est soutenue par une subvention de conférence des Instituts de recherche en santé du Canada (IRSC) et un financement de parrainage de la Society for Redox Biology and Medicine, de la Society for Free Radical Research - Europe, de la Biochimica Biophysica Acta-Molecular Cell Research journal, l'Institut Lady Davis pour la recherche médicale, l'Hôpital général juif, l'Université McGill – Faculté de médecine, la Fondation Armand Frappier, l'Université du Québec à Montréal, l'Université Concordia, les entreprises Roche, Nikon et Wisent, et la revue Antioxidants.
Pour inspirer l'imagination et offrir un répit aux présentations PowerPoint, aux salles de conférence et aux affiches, nous avons organisé un dîner de gala très spécial pour la cérémonie de remise des prix de cette année à l'hôtel Bonaventure le soir du vendredi 12 mai. Le soir du mardi 9, nous tiendrons un événement de réseautage et ``Brise glace`` au Hurley's Irish Pub, un authentique point de repère irlandais au cœur du centre-ville de Montréal depuis 1993 :
http://www.hurleysirishpub.com/en_home.html

Les participants à la conférence COSC 2023 sont invités à soumettre un article qui sera publié dans un numéro spécial de la conférence dans la revue Biochimica Biophysica Acta - Molecular Cell Research (BBAMCR). Le numéro spécial s'intitulera « Oxidative Stress in Health and Disease ». La date limite de soumission des articles pour le numéro spécial est le 30 novembre 2023. BBA Molecular Cell Research est l'une des dix revues thématiques de la série BBA de revues internationales. BBA - Molecular Cell Research se concentre sur la compréhension des mécanismes des processus cellulaires au niveau moléculaire.

Vous avez aussi des soirées libres pour visiter et explorer notre belle ville de Montréal, à distance de marche du Vieux-Montréal et du Vieux-Port, et d'autres quartiers du centre-ville à visiter et à explorer.

En vous remerciant pour votre participation et c'est avec beaucoup enthousiasme que nous vous accueillions au COSC 2023 !

Sincèrement,



Diana Averill-Bates, PhD
COSC 2023 Co-Chair
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Charles Ramassamy *(INRS-CAFSB)*
Ryan Mailloux *(McGill)*

Mardi / Tuesday, May 9, 2023

19h00 – 21h00 **Meet and Greet Introduction and Networking Event, Plus Registration**
Hurley's Irish Pub, 1225 Crescent St, Montreal, QC H3G 2B1

Mercredi / Wednesday, May 10, 2023

08h30 **Inscription / Registration –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

08h45 **Le mot de bienvenue / Opening Remarks – Welcome**

Chairs: **Diana Averill-Bates,** *UQAM - Université du Québec à Montréal, Montréal, QC CANADA* and
Kostas Pantopoulos, *Lady Davis Institute, McGill University, Montreal, QC CANADA*

Ernesto L. Schiffrin, C.M., MD, PhD, FRSC, FRCPC, FACP
Physician-in-Chief, Sir Mortimer B. Davis-Jewish General Hospital; Director, Hypertension and Vascular Research Unit, Lady Davis Institute for Medical Research; Distinguished James McGill Professor and Associate Chair; Department of Medicine, McGill University, Montreal, QC CANADA

CONFÉRENCIER D'HONEUR / PREMYSL PONKA KEYNOTE LECTURE 1

Chairs: **Kostas Pantopoulos and Sabrina Sgro,** *Lady Davis Institute, McGill University, Montreal, QC CANADA*

09h00 **Ferroptosis: From basic mechanisms to therapeutic opportunities.**
Marcus Conrad, *Helmholtz Zentrum München, Institute of Developmental Genetics, GERMANY*

10h00 **Pause santé / Break –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

PLENARY SESSION 1 – SYMPOSIUM IN HONOR OF DR PREMYSL PONKA IRON METABOLISM AND OXIDATIVE STRESS

Chairs: **Volker Blank and Wen Gu,** *Lady Davis Institute, McGill University, Montreal, QC CANADA*

10h20 **Tribute to Dr Premysl Ponka,** *Lady Davis Institute, McGill University, Montreal, QC CANADA*
Gerald Batist, *Deputy Director and Senior Investigator, Lady Davis Institute; Minda de Gunzburg, Professor of Oncology, McGill University; Director, Segal Cancer Centre, Sir Mortimer B. Davis-Jewish General Hospital; Director, McGill Centre for Translational Research in Cancer, Lady Davis Institute, Montreal, QC CANADA*
Kostas Pantopoulos, Volker Blank *(Lady Davis Institute, McGill University)* and former trainees of Dr Ponka

11h00 **The Janus face of non-transferrin bound iron (NTBI): Inducer of oxidative stress and regulator of the iron hormone hepcidin.**
Kostas Pantopoulos, *Lady Davis Institute, McGill University, Montreal, QC CANADA*

11h20 **Reactive oxygen species are an import mediator of the impact of excess iron on cardiometabolic disease.**
Gary Sweeney, *York University, Toronto, ON CANADA*

11h40 **Iron supplements modulate gut microbiota composition in mice.**
Manuela Santos, *Université de Montréal, Montréal, QC CANADA*

12h00 **Dîner / Lunch –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

**TRAINEE SYMPOSIUM 1*

Chairs: **Cat Grayson,** *McGill University, Montreal, QC CANADA*
Tetiana Shcholok, *University of Manitoba, Winnipeg, MB CANADA*
Moderator: **Ryan Mailloux,** *McGill University, Montreal, QC CANADA*

13h00 **Matrix metalloproteinase-2 inhibition prevents ER stress-mediated cell death during myocardial ischemia-reperfusion injury.**
***Wesman Bassiouni,** *University of Alberta, Edmonton, AB CANADA*

13h10 **Investigating the DEAD-box protein DDX28 and its role in metabolism and cancer.**
*Olivia Bebenek, *University of Guelph, Guelph, ON CANADA*

13h20 **The lysosomal TRPML3 channel fine-tunes inter-organellar communication to control proliferation and invasion of TNBC MDA-MB-231 cells.**
***Gabriela Gomes, *Dalhousie University, Halifax, NS CANADA*

13h30 **Sphingosine 1-phosphate signalling regulates phosphorylation and nitric oxide production during human sperm capacitation.**
*Steven Serafini, *McGill University, Montreal, QC CANADA*

13h40 **Maternal iron deficiency anemia and hypertension impairs renal mitochondrial respiration and elevates oxidative stress in kidneys**.
*Jad-Julian Rachid, *University of Alberta, Edmonton, AB CANADA*

13h50 **Acclimation and aging: Are they causally related?**
*Noah Lilienfeldt, *McGill University, Montreal, QC CANADA*

PLENARY SESSION 2 – REDOX CONTROL OF CELL SIGNALING

Chairs: **Cristian O'Flaherty and Steven Serafini,** *McGill University, Montreal, QC CANADA*

14h05 **Role of AKT/CREB signaling in ROS- induced expression of early growth response factor in vascular smooth muscle cells.**
Ashok Srivastava, *Université de Montréal*, *Montréal, QC CANADA*

14h25 **Novel insights in the regulation of redox signaling in spermatozoa.**
Cristian O'Flaherty, *McGill University, Montreal, QC CANADA*

14h45 **Mild heat shock induces an adaptive stress response: role of ROS and Nrf2.**
Diana Averill-Bates, *UQAM - Université du Québec à Montréal, Montréal, QC CANADA*

15h05 Pause santé / Break – Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

PLENARY SESSION 3 – OXIDATIVE STRESS RESPONSE NETWORKS – OMICS APPROACHES

Chairs: **Stefan Taubert and Judith (Junran) Yan,** *University of British Columbia, Vancouver, BC CANADA*

15h20 **Oxygen availability differentially regulates 8-oxoguanine placement in the genome.**
Jim Uniacke, *University of Guelph, Guelph, ON CANADA*

15h40 **Advances and challenges in the analysis of free-radical induced DNA damage by mass spectrometry.**
Richard Wagner, *Université de Sherbrooke, Sherbrooke, QC CANADA*

16h00 **Transcriptional regulation of oxidative stress response programs in *C. elegans* and in lung cancer cell lines.**
Stefan Taubert, *University of British Columbia, Vancouver, BC CANADA*

16h20 **Longitudinal studies of oxylipins using microsampling and *in vivo* microextraction.**
Dajana Vuckovic, *Concordia University, Montreal, QC CANADA*

16h40 **Integrated transcriptomics and proteomics analysis provides insight into the regulation of the abundance of specific proteins by vitamin C in the mouse liver.**
Michel Lebel, *Laval University, Quebec, QC CANADA*

POSTER SESSION 1

17h00-18h45 Séance d'affiches **/** Poster Session - **AMAR MAIN ENTRANCE – Next to JGH Block Amphitheatre**

SOIRÉE LIBRE / FREE EVENING

Jeudi / Thursday, May 11, 2023

08h30 **Inscription / Registration –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

PLENARY SESSION 4 – XENOBIOTICS AND TOXICOLOGY – LINKS TO OXIDATIVE STRESS

Chairs: **Arno Siraki and Newton Tran,** *University of Alberta, Edmonton, AB CANADA*

08h45 **Reactive metabolites of xenobiotics and their covalent binding to proteins studied by LC-MS/MS.**
Lekha Sleno, *UQAM - Université du Québec à Montréal, Montréal, QC CANADA*

09h05 **Tartrazine, curious and yellow - toxicology and metabolism of an Azo food dye.**
David Josephy, *University of Guelph, Guelph, ON CANADA*

09h25 **From antipsychotics to antiviral agents – Studies on diverse xenobiotic interactions with neutrophil myeloperoxidase.**
Arno Siraki, *University of Alberta, Edmonton, AB CANADA*

09h45 **Pause santé / Break –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

PLENARY SESSION 5 – CARDIOVASCULAR DISEASES

Chairs: **Madhu Anand-Srivastava,** *Université de Montréal, Montréal, QC Canada*
Palak Gujral, *Lady Davis Institute, Montreal, QC Canada*

10h05 **Sirtuin1 and regulation of blood pressure: Role of Gi proteins and nitroxidative stress.**
Madhu Anand-Srivastava, *Université de Montréal, Montréal, QC CANADA*

10h25 **Oxidative stress, inflammation and immune activation in hypertension.**
Ernesto L. Schiffrin, *Lady Davis Institute and Jewish General Hospital, McGill University, Montreal, QC CANADA*

10h45 **Identification of novel regulators of right ventricle hypertrophic remodeling with implications for pulmonary hypertension.**
Imad Al Ghouleh, *University of Pittsburgh School of* Medicine, *Pittsburgh, PA USA*

11h05 **ROS in vascular remodelling: going with the flow.**
Stephanie Lehoux, *Lady Davis Institute, McGill University, Montreal, QC CANADA*

11h25 **Matrix metalloproteinases as early effectors of oxidative stress injury.**
Rick Schulz, *University of Alberta, Edmonton, AB CANADA*

11h45 **Novel caffeine derivatives for the treatment of cardiovascular disease.**
Rick Austin, *McMaster University and St. Joseph's Healthcare, Hamilton, ON CANADA*

12h05 **Dîner / Lunch –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

CONFÉRENCIER D'HONEUR / KEYNOTE LECTURE 2

Chairs: **Charles Ramassamy and Hermine Counil,** *INRS-Centre Armand-Frappier-Santé Biotechnologie, Laval, QC CANADA*

13h15 **Oxidative stress: Eustress and distress.**
Helmut Sies, *Heinrich-Heine-University Düsseldorf, GERMANY*

**TRAINEE SYMPOSIUM 2*

Chairs: **Emily Poulton,** *University of Toronto, Toronto, ON Canada*
Cynthia Paz Trejo, *Université de Montréal, Montréal, QC Canada*
Moderator: **Kimberly Dunham-Snary,** *Queen's University, Kingston, ON Canada*

14h15 **Elucidation of a novel pathway of peroxyl radical trapping by ascorbate: Challenging the conventional electron transfer mechanism.**
*****Gabriel Robert, *Université de Sherbrooke, Sherbrooke, QC CANADA*

14h25 **Investigating the oxygen-dependent distribution and placement of oxidized guanines in human DNA and its impact on gene expression.**
*****Alexandria Kellington, *University of Guelph, Guelph, ON CANADA*

14h35 **Developmental depletion of neuronal thioredoxin-1 in mice results in structural and functional deficits.**
*Tetiana Shcholok, *University of Manitoba, Winnipeg, MB CANADA*

14h45 **The oxidation of fenamate compounds by neutrophil myeloperoxidase produces toxic reactive metabolites.**
*****Newton Tran, *University of Alberta, Edmonton, AB CANADA*

14h55 **efk-1/eEF2K mediates defense against starvation-induced oxidative stress in C. elegans.**
*****Judith (Junran Yan), *University of British Columbia, Vancouver, BC CANADA*

15h05 **Impact of reactive oxygen species on a cytosolic pattern recognition receptor: Nucleotide-binding, leucine rich repeat, pyrin containing 3 (NLRP3).**
*****Bjoern Ziehr, *University of Calgary, Calgary, AB CANADA*

15h15 **Pause santé / Break –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

*PLENARY SESSION 6 – OXIDATIVE STRESS RESPONSES IN PLANTS AND MICROORGANISMS, AND *TRAINEE SYMPOSIUM 3*

Chairs: **Angela Mungala Lengo,** *CHU Sainte-Justine, Université de Montréal, Montreal, QC Canada*
Si Ning Lui, *University of Alberta, Edmonton, AB Canada*
Moderator: ***Simon Labbé,*** *Université de Sherbrooke, Sherbrooke, QC CANADA*

15h35 **A new role for the peroxiredoxin Tpx1 and sulfiredoxin Srx1 as heme scavengers.**
Simon Labbé, *Université de Sherbrooke, Sherbrooke, QC CANADA*

15h55 **In vitro deglutathionylation of a cytosolic aldolase from *Arabidopsis thaliana*.**
*Charlie Boutin, *Université de Montréal, Montréal, QC CANADA*

16h05 **The story of Arabidopsis low expression of osmotically responsive genes 2: One locus, two proteins and multiple redox modifications.**
*Jasmine Ouellet, *Université de Montréal, Montréal, QC CANADA*

16h15 **Pseudophosphorylation of arabidopsis jasmonate biosynthesis enzyme lipoxygenase 2 via mutation of Ser600 inhibits enzyme activity.**
*Diljot Kaur, *Université de Montréal, Montréal, QC CANADA*

16h25 **Tuning the expression of Fe-S cluster machineries by a regulatory RNA.**
Éric Massé, *Université de Sherbrooke, Sherbrooke, QC CANADA*

POSTER SESSION 2

16h45-18h30 Séance d'affiches **/** Poster Session - **AMAR MAIN ENTRANCE – Next to JGH Block Amphitheatre**

SOIRÉE LIBRE / FREE EVENING

Vendredi / Friday, May 12, 2023

08h30 **Inscription / Registration –** Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

PLENARY SESSION 7 – NEURODEGENERATIVE DISEASES

Chairs: **Charles Ramassamy and Hermine Counil,** *INRS-Centre Armand-Frappier-Santé Biotechnologie, Laval, QC CANADA*

08h45 **Role of HO-1 in the pathogenesis of schizophrenia.**
Ayda Tavitian, *Lady Davis Institute, McGill University, Montreal, QC CANADA*

09h05 **Dual role of circulating exosomes in Alzheimer's disease: the vehicle for redox, inflammatory markers and the amyloid-peptide propagation or clearance?**
Charles Ramassamy, *INRS-Centre Armand-Frappier-Santé Biotechnologie, Laval, QC CANADA*

09h25 **Neuronal thioredoxin-1 depletion: from cellular deficits to widespread CNS degeneration.**
Eftekhar Eftekharpour, *University of Manitoba, Winnipeg, MB CANADA*

09h45 **Pause santé / Break -** Sponsored by the Chair Louise and André Charron on Alzheimer's Disease and the Foundation Armand-Frappier (FAF)

PLENARY SESSION 8 – OXIDATIVE STRESS IN AGING AND MITOCHONDRIAL DISEASE

Chairs: **Jeremy Michael Van Raamsdonk and Suleima Jacob-Thomas,** *McGill University, Montreal, QC CANADA*

10h05 **Targeting cardiolipin to alleviate mitochondrial redox and metabolic stress in Duchenne muscular dystrophy.**
Christopher Perry, *York University, Toronto, ON CANADA*

10h25 **Converting coenzyme Q from inefficient nutraceutical to an actual treatment option for coenzyme Q deficiency and other diseases.**
Siegfried Hekimi, *McGill University, Montreal, QC CANADA*

10h45 **Not just powerhouses anymore: Mitochondria are central hubs for cell redox signaling too.**
Ryan Mailloux, *McGill University, Montreal, QC CANADA*

11h05 **The complex relationship between oxidative stress and aging: How a mild increase in mitochondrial ROS can extend longevity.**
Jeremy Michael Van Raamsdonk, *McGill University, Montreal, QC CANADA*

CONFÉRENCIER D'HONEUR / KEYNOTE LECTURE 3

Chairs: **Siegfried Hekimi and Noah Lilienfeldt,** *McGill University, Montreal, QC CANADA*

11h30 **Redox regulation of RAS and RHO GTPases.**
Sharon Campbell, *Lineberger Cancer Center, University of North Carolina, NC USA*

12h30 Dîner / Lunch – Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

PLENARY SESSION 9 – OXIDATIVE STRESS IN CANCER

Chairs: **Volker Blank and Linda Yaker,** *Lady Davis Institute, McGill University, Montreal, QC CANADA*

13h30 **Linking CNC transcription factor function to stress signaling and inflammation.**
Volker Blank, *McGill University, Montreal, QC CANADA*

13h50 **Oxidative DNA damage, transcriptional regulation, senescence, and cancer cell adaptation.**
Alain Nepveu, *McGill University, Montreal, QC CANADA*

14h10 **Metabolic plasticity dictates oncogenic *versus* therapeutic functions of oxidative stress in breast cancer.**
Josie Ursini-Siegel, *McGill University, Montreal, QC CANADA*

14h40 **APP- and BACH1-dependent pathways: New avenues for modulating ferroptosis in cancer and neuronal ischemic injury.**
Vivek Venkataramani, *University Hospital Würzburg, Germany*

15h00 Pause santé / Break – Amar Main Entrance, Jewish General Hospital (Next to Block Amphitheatre B-106)

PLENARY SESSION 10 – OXIDATIVE STRESS IN HEALTH AND NUTRITION

Chairs: **Stan Kubow and Fang Lu,** *McGill University, Montreal, QC CANADA*

15h15 **Probiotics, the microbiome and the host gut immune system involving *Clostridioides difficile* infection.**
Stan Kubow, *McGill University, Montreal, QC CANADA*

15h35 **Oxidative stress and non-alcoholic fatty liver disease.**
Giada Sebastiani, *McGill University, Montreal, QC CANADA*

15h55 **Neutrophil mitochondria mediate killing of *Staphylococcus aureus ex vivo*.**
Kimberley Dunham-Snary, *Queen's University, Kingston, ON CANADA*

16h15 **Redox sensitive signaling adaptors control the efficiency of the antiviral response.**
Nathalie Grandvaux, *Université de Montréal, Montréal, QC CANADA*

16h35 **Air pollution exposure-related adverse health effects: Role of oxidative stress.**
Premkumari Kumarathasan, *Environmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON CANADA*

16h55 **Visualizing lipid peroxidation and electrophilic stress in cells.**
Gonzalo Cosa, *McGill University, Montreal, QC CANADA*

17h15 **END OF CONFERENCE**

19h00 - 23h00 **BANQUET AND AWARDS PRESENTATION**

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- First Prize - Prem Ponka Memorial Award
- Second Prize - Sponsored by *Biochimica Biophysica Acta - Molecular Cell Research*
- Third Prize - Sponsored by *Antioxidants*

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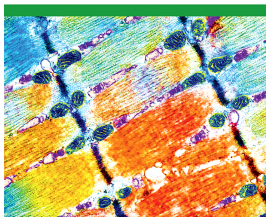

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antioxidants

Shan Soe-Lin, Prem Ponka memorial lecture and poster award

ORAL PRESENTATIONS in order of presentation 1 to 57

O-1 - KEYNOTE SPEAKER: MARCUS CONRAD
Helmholtz Zentrum Müchen
Institute of Development Genetics, Germany
Ferroptosis: From basic mechanisms to therapeutic opportunities

O-2 – KOSTAS PANTOPOULOS
Lady Davis Institute for Medical Research and
McGill University, Montreal, QC CANADA
The Janus face of non-transferrin bound iron (NTBI): Inducer of oxidative stress and regulator of the iron hormone hepcidin

O-3 – GARY SWEENEY
York University, Toronto, ON CANADA
Reactive oxygen species are an import mediator of the impact of excess iron on cardiometabolic disease.

O-4 – MANUELA M. SANTOS
CRCHUM / Université de Montréal
Montréal, QC CANADA
Iron supplements modulate gut microbiota composition and probiotic efficiency in mice

***O-5 – WESAM BASSIOUNI**
University of Alberta, Edmonton, AB CANADA
Matrix metalloproteinase-2 inhibition prevents ER stress-mediated cell death during myocardial ischemia-reperfusion injury

***O-6 – OLIVIA BEBENEK**
University of Guelph, Guelph, ON CANADA
Investigating the DEAD-box protein DDX28 and its role in metabolism and cancer

***O-7 – GABRIELA GOMES**
Dalhousie University, Halifax, NS CANADA
The lysosomal TRPML3 channel fine-tunes interorganellar communication to control proliferation and invasion of TNBC MDA-MB-231 cells.

***O-8 – STEVEN SERAFINI**
The Research Institute, MUCH
Montréal, QC CANADA
SPHINGOSINE 1-PHOSPHATE SIGNALLING REGULATES PHOSPHORYLATIONS AND NITRIC OXIDE PRODUCTION DUIRNG HUMAN SPERM CAPACITATION

***O-9 – JAD-JULIAN RACHID**
University of Alberta, Edmonton, AB CANADA
Maternal iron deficiency anemia and hypertension impairs renal mitochondrial respiration and elevates oxidative stress in kidneys.

***O-10 – NOAH LILIENFELDT**
McGill University, Montreal, QC CANADA
Acclimation and Aging: Are they causally related?

O-11 – ASHOK SRIVASTAVA
University of Montreal, CRCHUM
Montréal, QC CANADA
Role of AKT/CREB signaling in ROS- induced expression of early growth response factor in vascular smooth muscle cells

O-12 – CRISTIAN O'FLAHERTY
McGill University, The Research Institute-MUHC
Montreal, QC CANADA
Novel insights in the regulation of redox signaling in spermatozoa

O-13 – DIANA AVERILL-BATES
Université du Québec à Montréal
Montréal, QC CANADA
Mild heat shock induces an adaptive stress response: role of ROS and Nrf2

O-14 – JIM UNIACKE
University of Guelph, Guelph, ON CANADA
Oxygen availability differentially regulates 8-oxoguanine placement in the genome

O-15 – J. RICHARD WAGNER
Université de Sherbrooke, Sherbrooke, QC CANADA
Advances and challenges in the analysis of free-radical induced DNA damage by mass spectrometry

O-16 – STEFAN TAUBERT
University of British Columbia
Vancouver, BC CANADA
Transcriptional regulation of oxidative stress response programs in C. elegans and in lung cancer cell lines

O-17 – DAJANA VUCKOVIC
Concordia University, Montreal, QC CANADA
Longitudinal studies of oxylipins using microsampling and in vivo microextraction.

O-18 – MICHEL LEBEL
Concordia University, Montreal, QC CANADA
Integrated transcriptomics and proteomics analysis provides insight into the regulation of the abundance of specific proteins by vitamin C in the mouse liver

O-19 – LEKHA SLENO
UQAM, Université du Québec à Montréal
Montréal, QC CANADA
Reactive metabolites of xenobiotics and their covalent binding to proteins studied by LC-MS/MS

O-20 – DAVID JOSEPHY
University of Guelph, Guelph, ON CANADA
Tartrazine, Curious and Yellow - Toxicology and Metabolism of an Azo Food Dye

O-21 – ARNO SIRAKI
University of Alberta, Edmonton, AB CANADA
From antipsychotics to antiviral agents – studies on diverse xenobiotic interactions with neutrophil myeloperoxidase.

O-22 – MADHU ANAND-SRIVASTAVA
University of Montreal, Montreal, QC CANADA
SIRTUIN1 AND BLOOD PRESSURE REGULATION: ROLE OF GI PROTEINS AND NITROXIDATIVE STRESS

O-23 – ERNESTO L. SCHIFFRIN
Lady Davis Institute and Jewish General Hospital
McGill University, Montreal, QC CANADA
Oxidative stress, inflammation and immune activation in hypertension.

O-24 – IMAD AL GHOULEH
University of Pittsburgh School of Medicine
Pittsburgh, PA USA
Identification of Novel Regulators of Right Ventricle Hypertrophic Remodeling with Implications for Pulmonary Hypertension

O-25 – STEPHANIE LEHOUX
Lady Davis Institute, McGill University
Montreal, QC CANADA
ROS in vascular remodelling: Going with the flow

O-26 – RICHARD SCHULZ
University of Alberta, Edmonton AB CANADA
Matrix metalloproteinases as early effectors of oxidative stress injury

O-27 – RICHARD AUSTIN
McMaster University and St. Joseph's Healthcare
Hamilton, ON CANADA
Novel caffeine derivatives for the treatment of cardiovascular disease

O-28 – KEYNOTE SPEAKER: HELMUT SIES
Institute for Biochemistry and Molecular Biology I
Heinrich-Heine-University Düsseldorf, and Leibniz Research Institute for Environmental Medicine
Oxidative Stress: Eustress and Distress

***O-29 – GABRIEL ROBERT**
Université de Sherbrooke
Sherbrooke, QC CANADA
Elucidation of a novel pathway of peroxyl radical trapping by ascorbate: Challenging the conventional electron transfer mechanism

***O-30 – ALEXANDRIA KELLINGTON**
University of Guelph, Guelph, ON CANADA
Investigating the oxygen-dependent distribution and placement of oxidized guanines in human DNA and its impact on gene expression

***O-31 – TETIANA SHCHOLOK**
University of Manitoba, Winnipeg, MB CANADA
Developmental Depletion of Neuronal Thioredoxin-1 in mice results in structural and functional deficits

***O-32 – NEWTON TRAN**
Katz Group-Rexall Centre for Pharmacy
and Health Research
University of Alberta, Edmonton, AB CANADA
The Oxidation of Fenamate Compounds by Neutrophil Myeloperoxidase Produces Toxic Reactive Metabolites

***O-33 – JUDITH (JUNRAN) YAN**
University of British Columbia
Vancouver, BC CANADA
efk-1/eEF2K mediates defense against starvation-induced oxidative stress in C. elegans

***O-34 – BJOERN ZIEHR**
Libin Cardiovascular Institute
Cumming School of Medicine
University of Calgary, AB CANADA
IMPACT OF REACTIVE OXYGEN SPECIES ON A CYTOSOLIC PATTERN RECOGNITION RECEPTOR: NUCLEOTIDE-BINDING, LEUCINE RICH REPEAT, PYRIN CONTAINING 3 (NLRP3)

O-35 – SIMON LABBÉ
Université de Sherbrooke
Sherbrooke, QC CANADA
A new role for the peroxiredoxin Tpx1 and sulfiredoxin Srx1 as heme scavengers.

***O-36 – CHARLIE BOUTIN**
Institut de recherche en Biologie végétale
Université de Montréal, Montréal, QC CANADA
In vitro deglutathionylation of a cytosolic aldolase from Arabidopsis thaliana

***O-37 – JASMINE OUELLET**
Institut de recherche en biologie végétale
Université de Montréal, Montréal, QC CANADA
The story of Arabidopsis Low expression of Osmotically responsive genes 2: One locus, two proteins and multiple redox modifications

***O-38 – DILJOT KAUR**
Institut de recherche en biologie végétale
Université de Montréal, Montréal, QC CANADA
Pseudophosphorylation of Arabidopsis jasmonate biosynthesis enzyme lipoxygenase 2 via mutation of Ser600 inhibits enzyme activity

O-39 – ÉRIC MASSÉ
University of Sherbrooke
Sherbrooke, QC CANADA
Tuning the expression of Fe-S cluster machineries by a regulatory RNA

O-40 – AYDA TAVITIAN
McGill University, Lady Davis Institute for Medical Research, Jewish General Hospital
Montreal, QC CANADA
Heme oxygenase-1 in the Pathogenesis of Schizophrenia

O-41 – CHARLES RAMASSAMY
INRS-Centre Armand Frappier-Santé Biotechnologie
Laval, QC CANADA
Dual role of circulating exosomes in Alzheimer's disease: the vehicle for redox, inflammatory markers and the amyloid-peptide propagation or clearance?

O-42 – EFTEKHAR EFTEKHARPOUR
University of Manitoba
Winnepeg, MB CANADA
Neuronal Thioredoxin-1 depletion: from Cellular Deficits to widespread CNS degeneration.

O-43 – CHRISTOPHER PERRY
York University, Toronto, ON CANADA
Targeting mitochondrial cardiolipin to preserve bioenergetics and muscle health in Duchenne muscular dystrophy

O-44– SIEGFRIED HEKIMI
McGill University, Montreal, QC CANADA
Converting coenzyme Q from inefficient nutraceutical to an actual treatment option for coenzyme Q deficiency and other diseases

O-45 – RYAN J. MAILLOUX
McGill University
Ste-Anne-de-Bellevue, QC CANADA
Not just powerhouses anymore: mitochondria are central hubs for cell redox signaling too.

O-46 – JEREMY VAN RAAMSDONK
McGill University, Montreal, QC CANADA
The complex relationship between oxidative stress and aging: How a mild increase in mitochondrial ROS can extend longevity

O-47– KEYNOTE SPEAKER: SHARON CAMPBELL
Lineberger Cancer Center at the
University of North Carolina, Chapel Hill, NC USA
Redox Regulation of RAS and RHO GTPases

O-48 – VOLKER BLANK
Lady Davis Institute for Medical Research
Jewish General Hospital, McGill University
Montreal, QC CANADA
Linking CNC transcription factor function to stress signaling and inflammation

O-49 – ALAIN NEPVEU
Rosalind and Morris Goodman Cancer Institute
McGill University, Montreal, QC CANADA
Oxidative DNA Damage, Transcriptional Regulation, Senescence and Cancer Cell Adaptation

O-50 – Josie Ursini-Siegel
Lady Davis Institute for Medical Research, McGill University, Montréal, QC CANADA
p66ShcA-induced metabolic reprogramming maintains redox balance to promote the emergence of aggressive breast cancers.

O-51 – VIVEK VENKATARAMANI
University Medicine Göttingen
Robert-Koch-Strasse Göttingen, Germany
APP- and BACH1-Dependent Pathways: New Avenues for Modulating Ferroptosis in Cancer and Neuronal Ischemic Injury

O-52 – STAN KUBOW
McGill University, Montreal, QC CANADA
Probiotics, the microbiome and the host gut immune system involving Clostridioides difficile infection

O-53 – GIADA SEBASTIANI
McGill University Health Centre,
McGill University, Montreal, QC CANADA
Oxidative stress in non-alcoholic fatty liver disease

O-54 – KIMBERLY DUNHAM-SNARY
Queen's University, Kingston, ON CANADA
Neutrophil Mitochondria Mediate Killing of Staphylococcus Aureus ex vivo

O-55 – NATHALIE GRANDVAUX
CRCHUM, Université de Montréal
Montréal, QC CANADA
Redox sensitive signaling adaptors control the efficiency of the antiviral response.

O-56 – PREMKUMARI KUMARATHASAN
Health Canada
University of Ottawa, Ottawa, ON
Air pollution Exposure-Related Adverse Health Effects: Role of Oxidative stress

O-57 – GONZALO COSA
McGill University, Montreal, QC CANADA
Visualizing lipid peroxidation and electrophilic stress in cells

ORAL PRESENTATIONS
Speaker Abstracts in order of Presentation – 1 to 57

CONFÉRENCIER D'HONEUR / PREMYSL PONKA KEYNOTE LECTURE 1

O-1 - KEYNOTE SPEAKER: MARCUS CONRAD

Helmholtz Zentrum Müchen, Institute of Development Genetics, Germany

Ferroptosis: From basic mechanisms to therapeutic opportunities

Marcus Conrad[1]
[1]Helmholtz Munich Institute of Metabolism and Cell Death Ingolstädter Landstr. 1 D-85764 Neuherberg

Ferroptosis is a metabolic form of non-apoptotic cell death characterized by an iron-dependent oxidative destruction of cellular membranes[1]. Ferroptosis emerges as the underlying cause of a number of degenerative diseases, including neurodegeneration and tissue ischemia/reperfusion injury, and presents a pharmacologically tractable vulnerability to eradicate difficult-to-treat cancers. Nonetheless, formal proof of a physiological meaning of ferroptosis remains to be provided. We and others have shown that the selenoenzyme glutathione peroxidase 4 (GPX4) is the guardian of ferroptosis due to its unique activity to directly scavenge hydroperoxides in cellular membranes[2,3]. Using genetic suppressor screens we introduced the second mainstay in ferroptosis control, called ferroptosis suppressor protein-1 (FSP1), which can fully compensate for the loss-of GPX4[4]. The anti-ferroptotic role played by FSP1 is based on the NAD(P)H dependent reduction of extramitochondrial coenzyme Q_{10} (CoQ_{10}), thereby halting uncontrolled lipid peroxidation and ferroptosis. Moreover, we recently discovered a non-canonical vitamin K cycle driven by FSP1 as a novel mechanism that can efficiently protect against ferroptosis[5]. Besides its role in ferroptosis prevention, FSP1 was further found to be the long sought-after warfarin-resistant vitamin K reductase in the canonical vitamin K cycle[5], thus linking vitamin K biology and ferroptosis. Ongoing studies in our laboratory are therefore geared towards elucidating the in vivo relevance of the GPX4- and FSP1-dependent systems in the control of ferroptosis and their pharmacological tractability for disease prevention.

Further reading (*equal contribution)

1. Jiang X, Stockwell BR, Conrad M (2021) Ferroptosis: mechanisms, biology and role in disease. Nat Rev Mol Cell Biol. 2021 4:266-282.

2. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, Aichler M, Walch A, Eggenhofer E, Basavarajappa D, Rådmark O, Kobayashi S, Seibt T, Beck H, Neff F, Esposito I, Wanke R, Förster H, Yefremova O, Heinrichmeyer M, Bornkamm GW, Geissler EK, Thomas SB, Stockwell BR, O'Donnell VB, Kagan VE, Schick JA, Conrad M (2014) Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nat Cell Biol.16:1180-91.

3. Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, Buday K, Roveri A, Peng X, Porto Freitas F, Seibt T, Mehr L, Aichler M, Walch A, Lamp D, Jastroch M, Miyamoto S, Wurst W, Ursini F, Arnér ESJ, Fradejas-Villar N, Schweizer U, Zischka H, Friedmann Angeli JP, Conrad M (2018) Selenium Utilization by GPX4 Is Required to Prevent Hydroperoxide-Induced Ferroptosis. Cell. 172:409-422.e21.

4. Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, Grocin AG, Xavier da Silva TN, Panzilius E, Scheel CH, Mourão A, Buday K, Sato M, Wanninger J, Vignane T, Mohana V, Rehberg M, Flatley A, Schepers A, Kurz A, White D, Sauer M, Sattler M, Tate EW, Schmitz W, Schulze A, O'Donnell V, Proneth B, Popowicz GM, Pratt DA, Angeli JPF*, Conrad M* (2019) FSP1 is a glutathione-independent ferroptosis suppressor. Nature. 575:693-698.Mishima E*, Ito J, Wu Z, Nakamura T, Wahida A, Doll S, Tonnus W, Nepachalovich P,Eggenhofer E, Aldrovandi M, Henkelmann B, Yamada KI, Wanninger J, Zilka O, Sato E, Feederle R, Hass D, Maida A, Mourão ASD, Linkermann A, Geissler EK, Nakagawa K, Abe T, Fedorova M, Proneth B, Pratt DA, Conrad M* (2022) A non-canonical vitamin K cycle is a potent ferroptosis suppressor. Nature. 608:778-783.

5. Mishima E*, Ito J, Wu Z, Nakamura T, Wahida A, Doll S, Tonnus W, Nepachalovich P,Eggenhofer E, Aldrovandi M, Henkelmann B, Yamada KI, Wanninger J, Zilka O, Sato E, Feederle R, Hass D, Maida A, Mourão ASD, Linkermann A, Geissler EK, Nakagawa K, Abe T, Fedorova M, Proneth B, Pratt DA, Conrad M* (2022) A non-canonical vitamin K cycle is a potent ferroptosis suppressor. Nature. 608:778-783.

O-2 – KOSTAS PANTOPOULOS

Lady Davis Institute for Medical Research and McGill University, Montreal, QC CANADA

The Janus face of non-transferrin bound iron (NTBI): Inducer of oxidative stress and regulator of the iron hormone hepcidin

Kostas Pantopoulos[1]
[1]Lady Davis Institute for Medical Research and McGill University

Under physiological conditions, circulating iron is bound to transferrin, the plasma iron carrier. Transferrin delivers iron to tissues but also keeps the metal in a redox-inert state, preventing it from engaging into redox reactions that promote oxidative stress. In pathological states non-transferrin bound iron (NTBI) may emerge; this is readily taken up by tissue parenchymal cells, especially hepatocytes, and causes tissue damage. Hepatocytes are critical for systemic iron homeostasis by producing hepcidin, a peptide hormone that limits iron entry to the bloodstream by inactivating the iron exporter ferroportin in target tissues. Elevated body iron stores trigger transcriptionally induction of bone morphogenetic protein 6 (BMP6) in liver sinusoidal endothelial cells (LSECs). BMP6 is then secreted to activate SMAD signaling in neighboring hepatocytes, which culminates in transcriptional induction of the hepcidin-encoding *HAMP* gene. To explore the mechanism for iron sensing by LSECs, we generated $Tfrc^{Tek\text{-}Cre}$ mice with endothelial cell-specific ablation of transferrin receptor 1 (Tfr1). We also used control $Tfrc^{fl/fl}$ mice to characterize LSEC-specific molecular responses to iron by single-cell transcriptomics. $Tfrc^{Tek\text{-}Cre}$ animals tend to have modestly increased liver iron content (LIC) compared to $Tfrc^{fl/fl}$ controls but express physiological *Bmp6* and *Hamp* mRNAs. Despite a transient inability to upregulate *Bmp6*, they eventually respond to iron challenges with *Bmp6* and *Hamp* induction, yet occasionally to levels slightly lower relative to LIC. High dietary iron intake triggered accumulation of serum non-transferrin bound iron (NTBI), which significantly correlated with liver *Bmp6* and *Hamp* mRNA levels and elicited more profound alterations in the LSEC transcriptome compared to holo-transferrin injection. These culminated in robust induction of *Bmp6* and other nuclear factor erythroid 2-related factor 2 (Nrf2) target genes, as well as Myc target genes involved in ribosomal biogenesis and protein synthesis. LSECs and midzonal hepatocytes were the most responsive liver cells to iron challenges and exhibited highest expression of *Bmp6* and *Hamp* mRNAs, respectively. Our data suggest that during systemic iron overload, LSECs internalize NTBI, which promotes oxidative stress and thereby transcriptionally induces *Bmp6* via Nrf2. Thus, NTBI is not merely a toxic byproduct, but also serves as a pathophysiological regulator of iron homeostasis.

O-3 – GARY SWEENEY

York University, Toronto, ON CANADA

Reactive oxygen species are an import mediator of the impact of excess iron on cardiometabolic disease.

Gary Sweeney[1]
[1]Department of Biology, York University, Toronto, Canada.

Iron overload (IO) is associated with various pathological changes which contribute to heart failure, including cardiomyocyte insulin resistance and cell death. Treatment of primary adult and neonatal cardiomyocytes as well as H9c2 cells with iron decreased insulin sensitivity determined via Western blotting or immunofluorescent detection of Akt and p70S6K phosphorylation and glucose uptake. Cell death, occurring via intrinsic apoptosis pathway, was also increased after prolonged exposure to high levels of iron. Using CellROX deep red or DCF-DA probes we also observed that iron increased generation of reactive oxygen species (ROS). Pretreatment with MnTBAP, SKQ1 and allopurinol but not apocynin reduced iron-induced ROS suggesting mitochondria and xanthine oxidase contribute to cellular ROS in these cells upon iron excess. In particular, mitochondrial ROS were important in causing insulin resistance and cell death. We generated cells overespressing MitoNEET, an outer mitochondrial membrane protein involved in the transfer of Fe-S clusters between mitochondrial and cytosol, and observed lower iron in the mitochondria and ROS accumulation versus control cells. These alterations were correlated with reduced IO-induced cell death and insulin resistance in MitoNEET-overexpressing cells. Prolonged iron treatment attenuated autophagy flux, exacerbating ROS production. The cardioprotective hormone adiponectin or its receptor agonists can alleviate these effects of iron on insulin resistance and cell death. By generating autophagy-deficient cardiomyocytes we found that adiponectin signaling was protective at least in part by enhancing autophagy. In conclusion, IO mediates cardiomyocyte insulin resistance or cell death via mitochondrial iron accumulation and ROS upregulation.

O-4 – MANUELA M. SANTOS

CRCHUM / Université de Montréal, Montréal, QC CANADA

Iron supplements modulate gut microbiota composition and probiotic efficiency in mice

Marco Constante[1], Gabriela Fragosos[2], Annie Calvé[2], Manuela M Santos[1]
[1]CRCHUM/Université de Montréal, [2]CRCHUM

Almost all bacteria require iron for growth and survival. In order to successfully compete for this essential nutrient, bacteria developed very efficient iron uptake systems. Iron supplementation and iron fortification of food may impact the composition of the gut microbiota and exacerbate inflammation in inflammatory bowel disease (IBD).

We fed mice diets supplemented with ferrous sulphate at different doses (5, 50 and 500 mg of iron/Kg chow) and with different iron formulations (ferrous sulphate, ferrous bisglycinate, ferric ethylenediaminetetraacetic acid (FEDTA), and heme). We analyzed the effects on the composition of the gut microbiota by 16S ribosomal RNA gene sequencing. Using the dextran sodium sulphate (DSS)-induced colitis mouse model we investigated the effects of iron supplementation and iron supplementation in combination with the probiotic Escherichia coli Nissle 1917 (EcN) on colitis severity.

Iron supplementation with ferrous sulphate at different doses induced shifts in the gut microbial communities and had a protective effect on DSS-induced colitis. However, depending on the iron formulation used in the diets, iron supplementation in colitis was either beneficial (ferrous sulphate and ferrous bisglycinate) or highly detrimental (ferric EDTA and heme). Finally, the beneficial effect of the probiotic EcN in the DSS-induced colitis model was potentiated by oral iron supplementation.

These results suggest that the iron formulations used to treat iron deficiency may influence the gut microbiota and the severity of colitis. Additionally, the beneficial action of probiotics in IBD may be enhanced by oral iron supplementation.

*O-5 – WESAM BASSIOUNI

University of Alberta, Edmonton, AB CANADA

Matrix metalloproteinase-2 inhibition prevents ER stress-mediated cell death during myocardial ischemia-reperfusion injury

Wesam Bassiouni[1], Zabed Mahmud[2], John M. Seubert[1, 3], Richard Schulz[1, 2]
[1]Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, [2]Department of Pediatrics, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada, [3]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Objective: Matrix metalloproteinase-2 (MMP-2) is a ubiquitous protease that is activated intracellularly in response to oxidative stress during myocardial ischemia/reperfusion (IR) injury. Enhanced production of reactive oxygen-nitrogen species disrupts protein folding in the endoplasmic reticulum (ER) and induces ER stress. As a result the unfolded protein response (UPR) is activated through the stress sensor, inositol-requiring enzyme 1α (IRE1α), to restore protein folding. Failure of the UPR to reduce ER stress induces cellular dysfunction and apoptosis. MMP-2 localizes to the mitochondrial-ER-associated membrane. However, its role in ER homeostasis is unknown. We hypothesized that MMP-2 may proteolyze IRE1α during IR injury and stimulate the apoptotic response. We investigated whether MMP-2 inhibition could protect against ER stress-mediated cell death.

Methods and Results: Hearts from 3-month-old male mice subjected to in vitro IR injury (30 min global, no-flow ischemia followed by 40 min reperfusion) showed a significant reduction in left ventricular developed pressure (LVDP) compared to aerobically perfused controls. Ventricular extracts obtained from IR hearts at the end of reperfusion had higher levels of CHOP and cleaved caspases-3 and -9 in the cytosolic fraction and higher levels of Bax in the mitochondria-enriched fraction. Together, this indicates induction of ER stress-mediated apoptosis in IR hearts. IRE1α levels were reduced in IR injury by ~ 2-fold compared to aerobic controls, which correlated to the decreased LVDP ($p<0.05$). MMP-2 preferring inhibitors, ARP-100 (10 μM) or ONO-4817 (50 μM), given 10 min before ischemia, improved post-ischemic recovery of LVDP compared to IR hearts (% of pre-ischemic baseline at end of reperfusion: IR+vehicle 27.2±7.3, IR+ARP 54.1±3.0, IR+ONO 64.8±5.3%, $p<0.05$). Both ARP-100 and ONO-4817 attenuated the increase in CHOP, Bax and cleaved caspases and the reduction in IRE1α compared to IR hearts ($p<0.05$). In silico analysis of mouse IRE1α sequence showed several potential MMP-2 cleavage sites.

Conclusions: During myocardial IR injury, MMP-2 activation may impair the UPR response and induce apoptosis by proteolysis of IRE1α. Inhibition of MMP-2 activity protects against cardiac contractile dysfunction in part by preserving IRE1α and preventing ER stress-mediated cell death.

*O-6 – OLIVIA BEBENEK

University of Guelph, Guelph, ON CANADA

Investigating the DEAD-box protein DDX28 and its role in metabolism and cancer

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Hypoxia refers to a deficiency in oxygen delivery to tissues and has been associated with many pathophysiological processes, including cancer. Indeed, hypoxia is a characteristic of the tumour microenvironment, leading to increased metastasis, poor patient prognosis and resistance to treatments. Oxygen is required for oxidative phosphorylation (OXPHOS), which is used by eukaryotic cells to generate high levels of ATP. To produce energy, hypoxic cells must switch to the less efficient anaerobic glycolytic pathway. That said, a growing body of evidence demonstrates that OXPHOS is not completely shut down in tumours, and that the balance between glycolysis and OXPHOS utilization differs by cancer or cell type. We found that an RNA helicase, DDX28, suppresses the oncogenic HIF-2α axis, and is found at reduced levels in various tumors and cancer cell lines. We hypothesize that DDX28 is a tumour suppressor and is dysregulated in cancer. DDX28 is an RNA helicase belonging to the DEAD-box family and is involved in mitoribosome biogenesis by interacting with the mitochondrial 16S rRNA and the large subunit to assist with mitoribosome assembly. Because the mitoribosome is responsible for translating electron transport chain (ETC) proteins, many OXPHOS-related processes decrease upon DDX28 depletion, such as cell respiration, ETC complex activity and protein levels. We examined growth, invasion, and migration using U-87 MG human glioblastoma and MDA-MB-231 breast cancer 3D tumour models overexpressing DDX28. Previously, we have seen that total DDX28 protein levels decrease in hypoxia compared to normoxia (normal oxygen levels). Subcellular fractionation indicated that the greatest hypoxic decrease in DDX28 levels occurs in the mitochondria, while cytoplasmic DDX28 increases. Consequently, to begin investigating the mechanism by which DDX28 may be affecting cancer progression, the effect of DDX28 overexpression on the levels of several transcripts related to glycolysis and OXPHOS in normoxia and hypoxia was examined. We will also examine the effects of DDX28 dysregulation on other aspects of mitochondrial biology. Overall, this research will not only help define new functions of DDX28 and how it regulates cancer progression but will also contribute to the link between cell metabolism and cancer.

*O-7 – GABRIELA GOMES

Dalhousie University, Halifax, NS CANADA

The lysosomal TRPML3 channel fine-tunes interorganellar communication to control proliferation and invasion of TNBC MDA-MB-231 cells.

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Despite advances in diagnosis and cancer therapy, triple-negative breast cancer (TNBC) remains the deadliest subtype of breast cancer. This is largely because there are no effective therapeutic targets for TNBC and we do not understand the molecular basis of this disease. Increasing evidence suggests that lysosomes play a key role in the growth and therapy of TNBC tumors. However, it remains unclear which lysosomal proteins are important for TNBC proliferation and invasion. Using MDA-MB -231 cells as TNBC model and MCF10A control cells, we investigated the role of lysosomal TRPML3 (ML3) in TNBC cells. We show that knockdown of ML3 (ML3KD) reduces cell proliferation, induces G2/M cell cycle arrest, and promotes apoptosis and ROS /NO formation in TNBC. In addition, ML3KD reduces the motility, migration and invasion ability of TNBC. Mechanistically, ML3KD decreases the number/size of lysosomes but increases lysosomal acidification, ATP accumulation, and overall autophagy. These effects were accompanied by decreased mitochondrial oxygen consumption rate (OCR), decreased proton leak, and decreased OCR-ATP production. In conclusion, our research suggests a central role for ML3 in the interaction between lysosomes and mitochondria, which is essential for TNBC proliferation and invasion, and that inhibition of ML3 is likely an effective strategy against TNBC tumors.

*O-8 – STEVEN SERAFINI

The Research Institute, MUCH, Montréal, QC CANADA

SPHINGOSINE 1-PHOSPHATE SIGNALLING REGULATES PHOSPHORYLATIONS AND NITRIC OXIDE PRODUCTION DUIRNG HUMAN SPERM CAPACITATION

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Introduction & Objectives: ~15% of couples worldwide struggle with infertility, and ~50% of these cases are due to a male-related factor. The causes of male infertility remain unknown in 34% of cases. In addition, a semen analysis does not evaluate sperm capacitation. Sperm capacitation is a complex process the spermatozoon must undergo to acquire fertilizing ability. The role of lipid signalling in sperm capacitation is unknown. Bioactive sphingolipids (sphingosine (Sph), ceramide (Cer), and their phosphorylated forms S1P and C1P) promote nitric oxide ($NO^{\bullet}$) production in endothelial cells and macrophages. $NO^{\bullet}$ production is downstream of the PI3K/AKT pathway in sperm capacitation, but its regulation is unknown. We hypothesize that sphingolipids regulate tyrosine (P-Tyr) and PI3K (P-PI3K) phosphorylations and $NO^{\bullet}$ production needed for sperm capacitation. Our objectives are: 1) To determine whether sphingolipids promote sperm capacitation and the associated phosphorylations and $NO^{\bullet}$ production; 2) To assess sphingolipid metabolism and signalling pathways in human spermatozoa associated with sperm capacitation.

Methods: Human spermatozoa were incubated in BWW medium for 4hrs at 37°C, with or without the control inducer Fetal Cord Serum ultrafiltrate (FCSu), and Sph or Cer. Samples were capacitated with or without PF543, SLM 6031434, NVP231, VCP23019, TY-52156, L-NAME, chelerythrine, and U0126 (inhibitors of SPHK1, SPHK2, CERK, S1PR1, S1PR3, nitric oxide synthase (NOS), PKC, and MEK, respectively). P-Tyr (capacitation marker) and P-PI3K levels were determined by immunoblotting. S1P1R and phospho-SphK1 (P-SPHK1) were localized in spermatozoa using immunocytochemistry.

Results: Sph- and Cer-treated spermatozoa have increased capacitation compared to non-treated controls. Inhibition of SPHK1, CERK, and S1PR1 decreased P-Tyr and P-PI3K in capacitated spermatozoa compared to controls. Inhibition of S1PR3 and SPHK2 did not alter P-Tyr levels. S1PR1 and P-SPHK1 were localized in the post-acrosomal region in capacitated spermatozoa. P-SPHK1 was higher in capacitated samples and decreased with the inhibition of CERK. Inhibition of NOS decreased P-Tyr in sphingolipid-treated samples, indicating $NO^{\bullet}$ is generated downstream of the sphingolipid signalling cascade. Both PKC and MEK inhibitors decreased P-Tyr in Sph- and Cer-treated samples, thus are substrates downstream of S1P signalling.

Conclusions: S1P signalling is necessary to activate PKC, PI3K, ERK and subsequent $NO^{\bullet}$ production during capacitation, and its dysfunction could cause male infertility. These studies can drive the development of novel diagnostic tools and treatments for male infertility.

*O-9 – JAD-JULIAN RACHID

University of Alberta, Edmonton, AB CANADA

Maternal iron deficiency anemia and hypertension impairs renal mitochondrial respiration and elevates oxidative stress in kidneys.

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Introduction: Iron deficiency (ID) is the most common nutritional deficiency worldwide, affecting 40% of pregnant women. ID and anemia are modifiable risk factors for hypertensive disorders in pregnancy (HDP), although it is unclear how the former contributes to the pathophysiology of the latter. Both conditions are associated with oxidative stress, particularly within the kidney. The mitochondria are a major contributor to reactive oxygen species (ROS) generation within cells, and mitochondrial dysfunction is also a common etiological factor in ID and HDP. Here, we sought to determine whether dietary ID exacerbates mitochondrial dysfunction and oxidative stress in a rat model of hypertension in pregnancy.

Methods: Female spontaneously hypertensive rats (SHR) and normotensive control Wistar Kyoto rats (WKY) were fed either an iron-replete (37mg/kg) or an iron-restricted (3mg/kg) diet prior to and during pregnancy (n=4-8). Pregnant rats were euthanized on gestational day (GD) 21 (term=GD22). Maternal left kidneys were separated into medullary and cortical regions and homogenized for mitochondrial function assessments by high-resolution respirometry. Right kidneys were collected to assess gene expression profiles by RT-qPCR. Data were analyzed by two-way ANOVA followed by Holm-Sidak's posthoc test.

Results: ID reduced maternal hemoglobin (Hb) levels compared to control SHR/WKY counterparts ($p<0.0001$). Renal cortical mitochondrial respiration was decreased in both ID SHR and ID WKY. Specifically, ID SHR had a -9.4% and -11.3% decrease in NADH and succinate related pathways, respectively ($P=0.04$ and $P=0.01$). Medullary homogenates had reduced respiration in SHR compared to WKY in NADH, succinate and complex IV pathways ($P=0.005$, $P<0.0001$, and $P=0.007$, respectively). In SHR, ID increased gene expression of catalase (Cat) and glutathione peroxidase I (Gpx1), whereas the opposite effect was observed in ID WKY (both $P=0.04$). A similar interaction effect was observed in markers of apoptosis; ID SHRs had increased p53 expression (+42%, $P=0.0005$), while it was reduced in ID WKY (-20%, $P=0.001$).

Summary: ID led to reduced mitochondrial respiration in combination with oxidative imbalance in hypertensive pregnancies, which may lead to deleterious effects on cellular metabolism and survival. These results suggest that targeting the mitochondria in HDP could be an effective intervention strategy.

*O-10 – NOAH LILIENFELDT

McGill University, Montreal, QC CANADA

Acclimation and Aging: Are they causally related?

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Studies of long-lived clk-1 mutants in C. elegans have revealed a possible link between acclimation and aging. clk-1 encodes a mitochondrial hydroxylase required for the biosynthesis of ubiquinone, an essential electron transporter and likely antioxidant in the respiratory chain of the mitochondria. Ubiquinone deficiency in worms is associated with an extensive pleiotropic phenotype, characterized by defects in mitochondrial function, which result in decreased respiration and an altered redox state, as well as the slowing of physiological rates affecting development, behaviour, and aging. Mutations in clk-1 also cause abnormal responses to changes in temperature. In clk-1 mutants, the rates of embryonic development and defecation do not respond to changes in temperature, instead their rates remain typical of the initial temperature, at least for some time. These developmental and behavioural phenotypes reveal the existence of an active process of temperature compensation, which maintains physiological rates despite changes in temperature. This model also suggests that CLK-1 is required for sensing or reacting to temperature changes. The temperature unresponsiveness of the long-lived clk-1 mutants suggests the possibility of a link between acclimation and lifespan. We hypothesize that each time an animal acclimates to a new environment it runs the risk of not restoring a truly optimal physiology. Thus, the need to repeatedly adapt to a changing environment might lead to increased damage and dysregulation and contribute to aging or perhaps even cause aging. We are therefore wondering whether mutants that are unresponsive to changes in the environment might be long-lived under constant laboratory conditions because they do not (cannot) attempt constant, potentially damaging, adjustments to their physiology. To test this notion, we are exploring the relationship between temperature acclimation and the behaviour, development, and lifespan of clk-1 mutants, and have found that the rate of aging in clk-1 mutants also responds abnormally to changes in temperature. We are also investigating the effects of experimental “acclimative stress” on the survival of wild-type and mutant animals with impaired homeostasis and temperature sensation.

PLENARY SESSION 2 – REDOX CONTROL OF CELL SIGNALING

O-11 – ASHOK SRIVASTAVA

University of Montreal, CRCHUM, Montréal, QC CANADA

Role of AKT/CREB signaling in ROS- induced expression of early growth response factor in vascular smooth muscle cells

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Reactive oxygen species (ROS) are believed to play a key role in the pathophysiology of cardiovascular diseases. Excessive growth and proliferation of vascular smooth muscle cells (VSMC) has been suggested to be a major contributor to vascular dysfunction. Early growth response protein-1 (Egr-1), a zinc-finger transcription factor has been implicated in the development of vascular diseases. Recent studies have shown that hydrogen peroxide (H_2O_2), a ROS, increases Egr-1 expression in VSMC. However, signaling events leading to H_2O_2-induced Egr-1 expression are not fully understood. Therefore, here we aimed to determine signaling pathways implicated in H_2O_2-induced Egr-1 expression in VSMCs. We show that H_2O_2 enhanced the expression of Egr-1 which was associated with an increased phosphorylation of Akt. Pharmacological blockade of phosphatidylinositol-3-kinase (PI3K)/Akt pathway by wortmannin or SC66 significantly inhibited the protein and mRNA levels of Egr-1 induced by H_2O_2. H_2O_2-induced Egr-1 expression was associated with an increased phosphorylation of cyclic AMP response element binding protein (CREB), and pharmacological inhibition or the silencing of Akt attenuated both H_2O_2-induced CREB phosphorylation as well as Egr-1 expression. Moreover, RNA interference-mediated depletion of CREB almost completely suppressed the stimulatory effect of H_2O_2 on Egr-1 expression. In addition, pharmacological blockade or silencing of c-Src resulted in significant reduction in H_2O_2-induced Egr-1 expression as well as Akt and CREB phosphorylation. These data suggest that c-Src-mediated Akt and CREB -dependent signaling events mediate ROS-induced Egr-1 expression in VSMC (Supported by CIHR).

O-12 – CRISTIAN O'FLAHERTY

McGill University,The Research Institute-MUHC, Montreal, QC CANADA

Novel insights in the regulation of redox signaling in spermatozoa

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Spermatozoa are highly sensitive to high levels of reactive oxygen species (ROS). These active species oxidize lipids, proteins and DNA impairing vital functions of the spermatozoon and damaging the paternal genome with wide consequences for embryo development and promotion and developmental, cognitive and even fertility problems in the offspring. We found that peroxiredoxins (PRDXs), particularly PRDX6 are important antioxidant enzymes to protect spermatozoa against oxidative stress. All six members of the peroxiredoxin family are widely distributed in sperm compartments and are vital for the spermatozoon to maintain its viability, motility and ability to fertilize oocytes. Interestingly, spermatozoon must undergo a complex process called capacitation to be able to fertilize the oocyte. Sperm capacitation is highly sensitive to ROS since viable spermatozoa, exposed to ROS levels that affect motility, also impair capacitation.

On the other hand, low levels of ROS are essential for redox signaling during sperm capacitation. Superoxide anion, hydrogen peroxide, nitric oxide and peroxynitrite are needed to initiate and maintain capacitation. These ROS promote and timely regulate the activation of phosphorylation pathways during capacitation. Noteworthy, the ROS levels are kept low by PRDXs, to ensure redox signaling and avoid ROS-dependent toxic effects. Recently, we described the participation of PRDX6 peroxidase and calcium-independent phospholipase A_2 activities in the regulation of lipid signaling necessary to maintain sperm viability and ensure sperm motility and capacitation.

O-13 – DIANA AVERILL-BATES

Université du Québec à Montréal, Montréal, QC CANADA

Mild heat shock induces an adaptive stress response: role of ROS and Nrf2

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The exposure of cells to low doses of stress induces adaptive survival responses, which protect cells against subsequent exposures to cytotoxic stresses. These stresses include oxidative stress, hypoxia, radiation, heat shock, and heavy metals. The exposure of HeLa cells to mild stress (e.g. heat shock at 40°C) for an extended period can activate the cellular adaptive response and protect the cell against subsequent exposures to lethal stress doses (e.g. heat shock at 42°C, hydrogen peroxide). The ability of cells to resist subsequent toxic stress following exposure to low dose heat stress at 40°C is known as mild thermotolerance. Mild thermotolerance involves increased expression of heat shock proteins and antioxidants. However, the initiating factors in the antioxidant response are not entirely understood. Nuclear factor-erythroid factor 2-related factor 2 (Nrf2) is a redox-sensitive transcription factor that forms a cytosolic heterodimer with its inhibitor protein Keap1. Nrf2 is activated by reactive oxygen species (ROS) and translocates to the nucleus, where it binds to the antioxidant response element and upregulates expression of a multitude of antioxidant defenses. The role of Nrf2 in the cellular adaptive response is of the utmost importance. We aim to understand 1) the role of ROS generation in response to mild heat stress at 40°C in HeLa cells, and 2) the role of Nrf2 in the modulation of ROS generation and activation of the cellular adaptive response. Our results show that cellular ROS levels increase at 40°C and originate from both mitochondria and NADPH oxidase. Modulation of Nrf2 activity via overexpressor and knockdown cell lines significantly impacts levels of ROS generation during mild heat stress at 40°C. Elucidating the role of ROS in the induction of the cellular adaptive response is relevant to targeted hyperthermia treatments of cancer and could lead to new potential therapeutic treatments for a variety of diseases that implicate increased oxidative stress.

PLENARY SESSION 3 – OXIDATIVE STRESS RESPONSE NETWORKS – OMICS APPROACHES

O-14 – JIM UNIACKE

University of Guelph, Guelph, ON CANADA

Oxygen availability differentially regulates 8-oxoguanine placement in the genome

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[1]University of Guelph

Multicellular organisms balance oxygen delivery and toxicity by having oxygen pass through several barriers before cellular delivery. In human cell culture, these physiologic barriers are removed, exposing cells to higher oxygen levels. Human cells cultured in ambient air may appear normal, but this is difficult to assess without a comparison at physiologic oxygen. We examined the effects of culturing human cells throughout the spectrum of oxygen availability on oxidative damage to macromolecules, viability, proliferation, the antioxidant and DNA damage responses, metabolism, and mitochondrial fusion and morphology. We surveyed 4 human cell lines cultured for three days at seven oxygen conditions between 1 and 21% O_2. We show that oxygen levels and cellular benefit are not inversely proportional, but the benefit peaks within the physioxic range. Normoxic cells are in a perpetual state of responding to damaged macromolecules and mitochondrial networks relative to physioxic cells, which could compromise a research study. We also show that the 8-oxoguanine oxidative lesion has differential placements throughout the genome in response to changes in oxygen availability that is likely to influence gene expression. These data contribute to the concept of an optimal oxygen availability for cell culture in the physioxic range where the oxygen is not too high to reduce oxidative damage, and not too low for efficient oxidative metabolism, but just right: the Goldiloxygen zone.

O-15 – J. RICHARD WAGNER

Université de Sherbrooke, Sherbrooke, QC CANADA

Advances and challenges in the analysis of free-radical induced DNA damage by mass spectrometry

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Reactive oxygen and nitrogen species (ROS/RNS) generated by endogenous physiological processes lead to the formation of a plethora of DNA modifications-perhaps as many as 100 structurally distinct modifications including isomers. The repair of this damage is orchestrated by DNA repair pathways, such as base excision repair, which excise the damage and reinsert the cognate nucleotide. If the damage is not repaired properly or persists during cell division, the damage can be converted into mutations. The structure of damage ultimately determines the eventual recognition and kinetics of repair. Despite generally efficient DNA repair, it is believed that a steady state level exists in cellular DNA and thereby contributes to mutagenesis. Several biochemical and chemical methods have been developed to estimate the steady state levels of DNA damage in cells and tissues. Mass spectrometry (MS) can detect a multitude of different modifications with high sensitivity, and with unprecedented specificity, since it is based on the molecular and daughter ion masses used in tandem MS. Indeed, the type and distribution of DNA damage can point to the underlying pathways of damage in physiological terms. For example, certain ROS only induce the oxidation of guanine, and others, the nitration/halogenation of DNA bases. A major challenge to measure DNA damage by tandem MS analysis, particularly ROS-induced damage, is the possible occurrence of artificial oxidation during sample preparation. Two lesions that have caused considerable concern include 8-oxo-7,8-dihydroguanine and 5',8-cyclopurines. Tandem MS analysis of DNA damage is a powerful method if applied correctly; it can be used to measure DNA damage in model studies and certain endogenous damage, and validate other assays, such as the comet assay, and antibody and sequencing based assays.

O-16 – STEFAN TAUBERT

University of British Columbia, Vancouver, BC CANADA

Transcriptional regulation of oxidative stress response programs in C. elegans and in lung cancer cell lines

Stefan Taubert[1]
[1]University of British Columbia

Oxidative stress occurs during normal development and physiology as well as in diseases such as cancer and neurodegenerative disorders. To cope, organisms have evolved sophisticated adaptive mechanisms, which include transcriptional regulators that rewire gene expression to mitigate stress and repair cellular damage. The transcription factor Nrf2 is considered a master regulator in this response. However, the response to oxidative stress is complex and likely includes additional players. To identify genes and pathways that protect against oxidative stress, we are studying the model organism Caenorhabditis elegans, a nematode roundworm wherein many stress response regulators are conserved, including Nrf2 as SKN-1. Using genetic, molecular, and functional assays, we previously identified the Mediator subunit MDT-15/MED15 as a coregulator of SKN-1/Nrf2 in C. elegans. Interestingly, we additionally found that MDT-15 regulates some oxidative stress responsive genes in SKN-1 independent fashion, suggesting the existence of a parallel oxidative stress response pathway. Using a reverse genetic screen, we identified the nuclear hormone receptor NHR-49, an ortholog of mammalian peroxisome proliferator-activated receptor alpha (PPARa) and Hepatocyte Nuclear Factor 4 (HNF4), as a new oxidative stress response regulator. Additional genetic mapping identified several kinases and transcription factors that may also be part of this pathway. Mediator subunit MDT-15/MED15 therefore acts both with master regulator SKN-1/Nrf2 and with NHR-49/PPAR to ensure resilience against oxidative stress. Lastly, to test whether human MED15 also plays a role in the oxidative stress response, we inactivated it in the A549 lung adenocarcinoma cell line with siRNAs and by CRISPR/Cas9 mediated mutation. RNA-seq transcriptome profiling of these cells revealed reduced expression of genes regulated by Nrf2, including HO-1, a key enzyme that maintains cellular redox homeostasis. Our data thus suggest that MED15 is an evolutionarily conserved partner for Nrf2 and engages in new pathways to confer oxidative stress resilience.

O-17 – DAJANA VUCKOVIC

Concordia University, Montreal, QC CANADA

Longitudinal studies of oxylipins using microsampling and in vivo microextraction.

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Oxylipins are signalling lipids produced by oxidation of polyunsaturated fatty acids, either enzymatically using lipoxygenases, cyclooxygenases or cytochrome p450 enzymes or non-enzymatically via reaction with ROS. These lipid mediators play many important biological roles including regulation of inflammation and pain. The accurate measurement of oxylipins in biological fluids and tissues remains very challenging for several reasons, including poor stability, low concentrations and inability to adequately separate numerous isomers belonging to this family. In this talk, I will discuss two different approaches to perform longitudinal studies of oxylipins in rodent animal models in blood or tissue samples. First, I will discuss how in vivo solid-phase microextraction can be implemented using microdialysis cannula to directly sample oxylipins from brains in awake moving rats. This approach eliminates the need for tissue harvesting, allows complementary integration with microdialysis using a single set-up, and prevents well-documented artefact formation of oxylipins during tissue collection ifhead-focused microwave irradiation is not used. Our novel approach was able to accurately measure up to 52 eicosanoids and other oxylipins, and enable repeated sampling of the same animal over time with 23 oxylipins routinely detectable. Secondly, I will describe our solid-phase extraction liquid chromatography-mass spectrometry method for longitudinal measurement of oxylipins in murine blood using only 15 µL of plasma, and its application to study inflammatory processes during early development and progression of atherosclerosis in ApoE -/- mice fed low carbohydrate-high protein diet.

Overall, our novel methods can provide more accurate picture of oxylipin signalling and enable long-term longitudinal and/or in vivo monitoring for the first time.

O-18 – MICHEL LEBEL

Concordia University, Montreal, QC CANADA

Integrated transcriptomics and proteomics analysis provides insight into the regulation of the abundance of specific proteins by vitamin C in the mouse liver

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Vitamin C (ascorbate) is a crucial antioxidant and an essential cofactor of biosynthetic and regulatory enzymes. Unlike humans, mice can synthesize vitamin C thanks to the key enzyme gulonolactone oxidase (Gulo). Little is known about the molecular changes that take place in the liver during vitamin C deficiency. In the present study, we used the Gulo$^{-/-}$ mouse model, which cannot synthesize their own ascorbate to determine the impact of this vitamin on both the transcriptomics and proteomics profiles in the liver. The study included Gulo$^{-/-}$ mouse groups treated with either sub-optimal or optimal vitamin C concentrations in drinking water. We first observed that hepatic vitamin C levels in Gulo$^{-/-}$ mice increased proportionally to vitamin C concentrations in water. Secondly, we found a distinctive difference in the mRNA and protein profiles as a function of sex between all the mouse groups. Despite this sexual dimorphism, Spearman correlation analyses were conducted to divulge which transcripts and proteins correlated with hepatic vitamin C concentrations in both females and males. Such analysis on transcriptomics data from females and males revealed changes in a wide array of biological processes involved in glucose, lipid, steroid, and glutathione metabolisms as well as in acute-phase response. The hepatic levels of few of these proteins associated with such biological processes correlated with hepatic vitamin C levels. Furthermore, although several proteins of the mitochondrial complex III significantly correlated with vitamin C concentrations, their corresponding transcripts did not correlate with vitamin C in both females and males. Such observations were confirmed by western blot and quantitative RT-PCR analyses, respectively. Concomitantly, liver of vitamin C-deficient Gulo$^{-/-}$ mice exhibited lower ATP levels and increased reactive oxygen species. Thus, this side-by-side comparison between transcriptome and proteome supported the view that post-transcriptional processes play a major role in the regulation of cellular respiration in the liver upon vitamin C deficiency. Our findings suggest that transcriptome profiling alone provides an incomplete picture of molecular changes associated with vitamin C deficiency in the liver of mice and examination of changes in protein abundances is essential to unveil variations that are not transcriptionally regulated.

PLENARY SESSION 4 – XENOBIOTICS AND TOXICOLOGY – LINKS TO OXIDATIVE STRESS

O-19 – LEKHA SLENO

UQAM, Université du Québec à Montréal, Montréal, QC CANADA

Reactive metabolites of xenobiotics and their covalent binding to proteins studied by LC-MS/MS

Lekha Sleno[1]
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Many xenobiotics, when metabolized, can form reactive species for oxidative biotransformation by CYPs. This presentation will discuss methods of forming these species using in vitro incubations with liver fractions and their analysis by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Examples will be discussed from the metabolism of well-known drugs and environmental contaminants. Using acetaminophen as a model hepatotoxin, the identification of specific sites of covalent modifications on proteins in the liver has also been achieved with developed methods from our group. We have also developed a quantitative assay to assess amount of covalent binding in plasma samples occurring in rat and humans after APAP exposure.

O-20 – DAVID JOSEPHY

University of Guelph, Guelph, ON CANADA

Tartrazine, Curious and Yellow - Toxicology and Metabolism of an Azo Food Dye

David Josephy[1]
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Tartrazine (Yellow 5; E102), an anionic azo-pyrazolone synthesized in 1887, is found in a vast range of processed foods, beverages, and personal care products. Dietary intake of tartrazine by children may be >1 mg/kg bw/d. Although its use is permitted here and, in the USA, tartrazine is banned in some European countries.

An association between tartrazine consumption and behavioural effects, such as hyperactivity, has long been suspected. A recent comprehensive review by the California Office of Environmental Health Hazard Assessment (M.D. Miller et al., Environ. Health, 2022) concluded that “the scientific literature supports an effect of synthetic food dye exposures on neurobehavior in children at environmentally relevant exposure levels.” The authors also noted that “the breakdown of azo dyes in the gut prior to absorption requires toxicological examination of metabolites.”

Based mainly on rodent studies, several research groups have ascribed neurological and behavioural effects of tartrazine to oxidative stress, but the pertinence of this mechanism remains unproven.

Surprisingly little is known concerning the identity of the tartrazine metabolites formed - mainly by the gut microbiome - following oral ingestion. Tartrazine reduction releases two products, sulfanilic acid and ‘SCAP’, (1-p-sulfophenyl-3-carboxy-4-amino-5-pyrazolone). In 1965, Westöö observed that rats fed tartrazine eliminated a violet-coloured product in the feces, and she suggested its structure to be a pyrazolone dimer analogous to rubazoic acid, formed from SCAP. We have synthesized this product and verified its structure, and we are studying its potential toxicity.

We have identified species of the human gut microbiome which carry out the reduction of tartrazine. Characterizing the azoreductases from these bacteria will inform our studies of tartrazine metabolism.

O-21 – ARNO SIRAKI

University of Alberta, Edmonton, AB CANADA

From antipsychotics to antiviral agents – studies on diverse xenobiotic interactions with neutrophil myeloperoxidase.

Arno Siraki[1]
[1]University of Alberta

Neutrophils have a plethora of mechanisms that are activated upon appropriate stimulation. While many of these pathways are geared towards eliminating pathogens, there are cases to be made for the effects these mechanisms can have on xenobiotics. This presentation will cover the impact of neutrophil myeloperoxidase, isolated or within neutrophils, and the respiratory burst on various xenobiotics, including many drugs. It is well known the myeloperoxidase metabolism of xenobiotics generates free radical metabolites. However, we will present cases where there is an overlap between cytochrome P450 and myeloperoxidase. In addition, the pharmacological and toxicological consequences of these reactions will be discussed. Recent studies in the laboratory are focused on antiviral nucleoside analogs used for treating COVID-19. Although neutrophils are not the main target of these antiviral agents, they are involved in acute inflammatory and immune responses. Drugs including remdesivir, molnupiravir, favipiravir, and related metabolites will be discussed with respect to their interactions with neutrophils and neutrophil myeloperoxidase to present a potential scenario of the drugs’ fate during an acute infection.

PLENARY SESSION 5 – CARDIOVASCULAR DISEASES

O-22 – MADHU ANAND-SRIVASTAVA

University of Montreal, Montreal, QC CANADA

SIRTUIN1 AND BLOOD PRESSURE REGULATION: ROLE OF GI PROTEINS AND NITROXIDATIVE STRESS

Madhu Anand-Srivastava[1]
[1]University of Montreal

We recently showed that vascular smooth muscle cells (VSMC) from SHR exhibit overexpression of Sirtuin1 (Sirt1) that contributes to the enhanced expression of Giα proteins implicated in the development of hypertension in SHR. The present study investigated if the inhibition of Sirt1 could also ameliorate hypertension in SHR and explore the underlying molecular mechanisms. For this study, a selective inhibitor of Sirt1, EX-527 (5mg/kg of body weight), was injected intraperitoneally into 8-week-old SHR and age-matched WKY rats twice per week for 3 weeks. The blood pressure (BP) and heart rate was measured twice a week by the CODA™ non-invasive tail cuff method. The high BP and augmented heart rate in SHR was significantly attenuated by EX-527 treatment which was associated with the suppression of the overexpression of Sirt1 and Giα proteins in heart, VSMC and aorta. In addition, the enhanced levels of superoxide anion, NADPH oxidase activity, overexpression of NADPH oxidase subunits and FOXO1 were attenuated and the decreased levels of endothelial nitric oxide synthase (eNOS), nitric oxide (NO) and increased levels of peroxynitrite ($ONOO^-$) and tyrosine nitration in VSMC from SHR were restored to control levels by EX-527 treatment. Furthermore, knockdown of FOXO1 by siRNA also attenuated the overexpression of Giα-2 and NADPH oxidase subunit proteins and restored the decreased expression of eNOS in VSMC from SHR. These results reveal that the inhibition of overexpressed Sirt1 and its target FOXO1 through decreasing the enhanced levels of Giα proteins and nitro-oxidative stress attenuates the high BP in SHR. It may thus be suggested that inhibitors of Sirt1 may have the potential to be used as therapeutic agents in the treatment of cardiovascular complications associated with hypertension. (Supported by grant from CIHR)

O-23 – ERNESTO L. SCHIFFRIN

Lady Davis Institute and Jewish General Hospital, McGill University, Montreal, QC CANADA

Oxidative stress, inflammation and immune activation in hypertension.

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Angiotensin II, aldosterone and endothelin are important mediators leading to elevation of blood pressure and target organ damage in hypertension. Angiotensin II via membrane AT1 receptors, endothelin via ETA and ETB receptors, and aldosterone via genomic and non-genomic mechanisms activate NADPH oxidase to induce generation of superoxide anion. The latter is involved in the activation of inflammatory mechanisms. Stimulation of immune cells by these major mediators involved in hypertension leads as well to oxidative stress in the vascular wall. We have identified macrophages and monocytes from the innate immune system, and gamma/delta lymphocytes that are unconventional and innate like cells, as some of the first responders in the immune mechanisms associated with blood pressure elevation, inflammation and oxidative stress in the vascular wall and the kidney in experimental murine hypertension. Some of these mechanisms may become future targets for therapy in hypertension. (Supported by CIHR Foundation Grant 143348.)

O-24 – IMAD AL GHOULEH

University of Pittsburgh School of Medicine, Pittsburgh, PA USA

Identification of Novel Regulators of Right Ventricle Hypertrophic Remodeling with Implications for Pulmonary Hypertension

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Introduction: The molecular mechanisms underlying right ventricle (RV) hypertrophic remodeling (both adaptive and maladaptive) in response to sustained pressure overload, such as that experienced during pulmonary hypertension (PH), are inadequately understood. We previously implicated the cytosolic NADPH oxidase (Nox) organizer protein $p47^{phox}$ in pressure overload-induced RV hypertrophy. We have also implicated the Nox1 oxidase and Nox1-derived reactive oxygen species (ROS) in hypertrophic responses in vascular cells. However, whether Nox1 plays a role in RV remodeling under stress is inadequately explored.

Methods and Results: Cardiomyocyte-derived H9C2 cells and RV rat neonatal cardiomyocytes isolated from 1-day-old pup hearts (RV-RNCM) were subjected to neurohormonal hypertrophic stimulation using angiotensin II (AngII, 1 & 10 mM). RV pressure overload was induced in mice by pulmonary artery banding (PAB; 3wk). AngII treatment resulted in H9C2 and RV-RNCM hypertrophy and induced Nox1 protein expression. The AngII-induced hypertrophy was attenuated by Nox1 gene knockdown using siRNA. Inhibition of Nox1 with the isoform-specific peptidic inhibitor, NoxA1ds, attenuated AngII-induced hypertrophy in RV-derived but not left ventricle (LV)-derived cardiomyocytes, supporting heart-chamber preference. In vivo, PAB increased RV Nox1 expression and ROS. Nox1 null mice were protected from PAB-induced RV hypertrophy as indicated by the Fulton Index (ratio of RV to LV+septum weights). Finally, Nox1 expression and ROS were increased in RV tissue from PH patients vs. non-PH controls.

Conclusion: The present study supports a role for Nox1 in mediating pro-hypertrophic cellular responses in cardiomyocytes and identify potential therapeutic targets for RV dysfunction in PH.

O-25 – STEPHANIE LEHOUX

Lady Davis Institute, McGill University, Montreal, QC CANADA

ROS in vascular remodelling: Going with the flow

Stephanie Lehoux[1]
Lady Davis Institute, McGill University

----- NO ABSTRACT AVAILABLE -----

O-26 – RICHARD SCHULZ

University of Alberta, Edmonton AB CANADA

Matrix metalloproteinases as early effectors of oxidative stress injury

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Oxidative stress plays a fundamental role in both normal physiology and in pathological processes. Oxidative stress is manifested through the production of reactive oxygen-nitrogen species (RONS) an inclusive term which encompasses the key roles of nitric oxide and superoxide, and thereby peroxynitrite, in the generation of several more reactive molecules as effectors of oxidative stress. Cysteine sulphydryl moieties on proteins are readily oxidized by RONS to reversible and irreversible post-translational modifications, leading to activation or inactivation of protein function. Matrix metalloproteinases (MMPs) are a family of proteases which can be activated by low concentrations of peroxynitrite by S-glutathiolation of a key cysteine residue. Although MMPs were originally thought only to be secreted proteins activated in the extracellular space, in the past 25 years they were discovered to be localized to several subcellular compartments where upon RONS activation they proteolyze a rapidly growing number of discrete intracellular protein targets to effect several biological functions. MMP-2, one of 23 human MMPs, is found in nearly all cells and the central theme of my research. My talk will focus on the relationship of oxidative stress to activation of MMP-2 and intracellular proteolysis of its targets. This occurs on a minutes time scale in response to oxidative stress. MMP-2 is a very early effector of oxidative stress injury and a biomarker of oxidative stress, in whichever biological system you may be studying. The worlds of oxidative stress and proteolysis are thereby intertwined at a molecular level. A better understanding of this will help in the development of a novel class of therapeutics, intracellular MMP inhibitors, which have potential to be of wide utility against several diseases caused by enhanced oxidative stress.

O-27 – RICHARD AUSTIN

McMaster University McMaster University and St. Joseph's Healthcare, Hamilton, ON CANADA

Novel caffeine derivatives for the treatment of cardiovascular disease

Richard Austin[1], Jakob Magolan[1], Guillaume Paré[1], Paul Lebeau[1], Derek Stouth[1], Matthew Sguazzin[1]
[1]McMaster University

There is accumulating clinical evidence that caffeine (CF), one of the most highly consumed naturally occurring drugs found in coffee and tea, reduces cardiovascular disease (CVD) risk. The average adult consumes between 400 and 600 mg/day of CF and organizations like Health Canada and the Food and Drug Administration conclude that such doses are not negatively associated with toxicity, cardiovascular effects, bone status, or incidence of cancer. On the contrary, moderate to high levels of CF, consumed daily in the form of non-alcoholic beverages, are associated with a protective outcome on the cardiovascular system. Despite these recent findings, the underlying mechanism by which CF protects against CVD is not completely understood. We have now reported for the first time how CF protects against CVD. In the liver, CF is able to indirectly reduce the transcriptional activation of the sterol regulatory-element binding proteins (SREBP1/2), thereby increasing the expression of genes involved in the de novo synthesis and uptake of lipids which contribute to atherosclerosis, the underlying cause of CVD. Specifically, inhibition of SREBP2 reduces the blood levels of PCSK9, a circulating factor responsible for degrading the LDL receptor on the surface of liver cells. As a result, LDL receptor levels are dramatically increased, leading to a reduction in atherogenic lipids, including LDL cholesterol and triglycerides. Based on our findings, we have now developed a wide range of unique CF derivatives that have greater potency in blocking SREBP1/2 activation and reducing blood PCSK9 levels, compared to CF alone. We predict that these CF derivatives could represent new class of medicines for the treatment of CVD and its metabolic disturbances, including diabetes, obesity and fatty liver disease.

CONFÉRENCIER D'HONEUR / KEYNOTE LECTURE 2

O-28 – KEYNOTE SPEAKER: HELMUT SIES

Institute for Biochemistry and Molecular Biology I, Heinrich-Heine-University Düsseldorf, and Leibniz Research Institute for Environmental Medicine

Oxidative Stress: Eustress and Distress

Helmut Sies[1]
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In 1936 Hans Selye, at McGill University, introduced the "syndrome produced by diverse nocuous agents" into the life sciences [1]. Since then, stress research has richly developed into the psychosocial field and into biochemistry/biology/biomedicine. In the molecular organisation of cell metabolism, redox reactions are linked to fundamental life processes, with the main flux in aerobic metabolism going from reduced substrates to oxidized products. A steady-state redox tone is maintained in redox homeostasis, and a disbalance towards oxidation is termed "oxidative stress" [2, 3]. Recent research revealed a central role of hydrogen peroxide (H_2O_2) in redox regulation and oxidative stress responses. A physiological low level of H_2O_2 is essential in redox signaling, "oxidative eustress", whereas supraphysiological H_2O_2 is detrimental, causing molecular damage, "oxidative distress" [4]. Fine-tuning H_2O_2 steady-states in specific cell-types and subcellular organelles represents a challenge for a future redox medicine [5][6].

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*O-29 – GABRIEL ROBERT

Université de Sherbrooke

Elucidation of a novel pathway of peroxyl radical trapping by ascorbate: Challenging the conventional electron transfer mechanism

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[1]Université de Sherbrooke

Small-molecule antioxidants are crucial to the viability of cells that are continuously exposed to damaging radical species. The radical scavenging proprieties of ascorbate (Asc; vitamin C) are conventionally attributed to its efficiency to undergo single-electron transfers with reactive species. According to this mechanism, the specific reaction between ascorbate and biologically relevant peroxyl radicals results in the formation of hydroperoxides (ROOH). However, we uncovered contradicting evidence suggesting the existence of a novel reaction pathway in which the formation of ROOH is bypassed, ultimately affording alcohol products (ROH). To better understand this phenomenon, we investigated the reactivity of Asc with a thymidine peroxyl radical that was selectively generated using a photocleavable precursor. Detailed mechanistic studies and product analysis by LC/MS-MS revealed that ROH formation increased as a function of Asc concentration with concomitant decrease of ROOH. By examining the specific one-electron reduction chemistry of ROOH into intermediate alkoxyl radicals, we showed that these results cannot be explained by consecutive electron transfers. Instead, we propose that peroxyl radicals undergo addition to the ene-diol portion of Asc, followed by O_2-oxidation and Baeyer-Villiger rearrangement. Although Asc is destroyed in this process, the formation of hydroperoxides that can initiate additional radical reactions is prevented. Our study clarifies fundamental antioxidant mechanisms and challenges the current understanding that ascorbate acts as a radical-trapping agent solely because of its one-electron reducing potential.

*O-30 – ALEXANDRIA KELLINGTON

University of Guelph

Investigating the oxygen-dependent distribution and placement of oxidized guanines in human DNA and its impact on gene expression

Alexandria Kellington[1], Ryan Gillett[1], James Uniacke[1]
[1]University of Guelph

Culturing cells in the ambient oxygen concentration of 21% (160 mmHg; normoxia) is common practice, despite the reality that most mammalian cells exist within the body at oxygen concentrations ranging from 2-12% (15-92 mmHg; physioxia). In tissue culture, cells present healthier phenotypes in the physioxic range in comparison to normoxia or at deficient oxygen levels (1% O_2, 7.5 mmHg; hypoxia). The different phenotypes observed in normoxia, physioxia, and hypoxia are a result of altered gene expression and suggest different transcriptomes in response to oxygen availability. One oxygen-sensitive mechanism that has the potential to impact transcription does so due to the presence of oxidized nucleotides within DNA promoters. Specifically, recent studies have shown that the incorporation of oxidized guanines (8-oxoG) within gene promoters can increase gene expression through the recruitment of base excision repair (BER) proteins and, subsequently, transcriptional machinery. When mapped to the genome, 8-oxoG placement is non-random and are preferentially distributed to promoters. However, it is still unclear how oxygen concentration can impact promoter oxidation. Here, we have paired an immunoprecipitation method to isolate DNA containing 8-oxoG in human cell culture with genome-wide sequencing. First, gene promoters were confirmed to be preferentially oxidized relative to the rest of the genome, with protein-coding promoters oxidized similarly to other promoter types. However, normoxic, physioxic, and hypoxic genomes differed in which protein-coding promoters were oxidized. Finally, we found that the levels of 8-oxoG in some of these uniquely oxidized promoters correlate with an increase of gene expression. Ultimately, we reveal the potential for 8-oxoG to be an oxygen sensitive transcriptional switch to impact gene expression. This work will elucidate how physioxic gene expression differs from conventional normoxic cell culture and expand our knowledge of how oxygen regulates genes.

*O-31 – TETIANA SHCHOLOK

University of Manitoba, Winnipeg, MB CANADA

Developmental Depletion of Neuronal Thioredoxin-1 in mice results in structural and functional deficits

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Neuronal laminopathy (NLP) is newly identified feature in neurodegenerative diseases. NLP is induced by damage to nuclear lamina, a protein network at the interface of inner nuclear envelope and chromatin, with importance in gene regulation. NLP is detectable in most hippocampal neurons from autopsy samples in Alzheimer's disease (AD) patients. We recently discovered that NLP is mediated by activation of caspase-6 after downregulation of Thioredoxin -1 protein (Trx1). Trx1 is a major regulator of redox balance responsible for rejuvenating oxidized proteins and is reduced in autopsy from AD patients.

Using a Cre-recombinase system we developed a mouse model harboring a neuron specific Trx1-deletion. These animals survive up to 10 weeks and show signs of movement deficits. Here, we examined the cellular and molecular changes in hippocampus from 8-week-old mice and confirmed that induction of NLP in hippocampal neurons was associated with significant decrease in Trx1 and increased Tau phosphorylation and total amyloid beta in these mice. Trx-1 deficient mice also contained elevated levels of phosphorylated TDP-43 (Tar DNA binding protein 43); a marker associated with neurodegeneration. Evidence of astrocyte and microglia activation as well as oligodendrocyte damage and demyelination were observed. These animals also showed lower neurogenesis capacity in the adult-derived neural stem cells from Dentate Gyrus. Our study provides a direct link between decreased antioxidative capacity with development of neurodegeneration characteristic markers.

***O-32 – NEWTON TRAN**

Katz Group-Rexall Centre for Pharmacy and Health Research, University of Alberta, Edmonton, AB CANADA

The Oxidation of Fenamate Compounds by Neutrophil Myeloperoxidase Produces Toxic Reactive Metabolites

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Purpose: The interactions between peroxidase enzymes and fenamic acid-like NSAIDs cause the formation of reactive oxygen species, potentially leading to toxic side-effects. The aim of this study was to investigate the bioactivation of arylamine-containing fenamates based on N-phenylanthranilic acid (NPA) and four drug analogues: flufenamic acid (FFA), mefenamic acid (MFA), meclofenamic acid (MCFA), and tolfenamic acid (TFA) via myeloperoxidase (MPO). The heme enzyme MPO is known to catalyze oxidation reactions of numerous xenobiotics into reactive metabolites. We hypothesized that the enzymatic oxidation of these fenamates by MPO/H_2O_2 will result in reactive metabolites that cause oxidative damage and induce toxicity in leukemia cells. **Methods:** To test our hypothesis, we used biochemical approaches where purified MPO from human neutrophils was used for UV-vis spectrophotometry, liquid chromatography-mass spectrometry (LCMS), and electron paramagnetic resonance (EPR). In addition, in vitro studies were performed with HL-60 promyelocytic leukemia cells, which are high in MPO content, to analyze the cytotoxic potential of these reactive metabolites. We also used anti-DMPO antibody to detect DMPO (5,5-dimethyl-1-pyrroline N-oxide) covalently bound to protein, which forms only by the reaction of DMPO with a protein free radical. Results: In UV-vis spectrophotometry studies, it was observed that MPO catalyzed the oxidation of most fenamates, but not NPA. LCMS analysis of the oxidized products displayed the formation of dimers, hydroxylated, and quinoneimine species, although, glutathione (GSH) conjugates were only detected for MFA and TFA. EPR spin trapping with DMPO using reduced GSH revealed that all fenamates produced glutathionyl radicals ($GS^{\bullet}$) in a linear concentration-dependent manner, with the highest and lowest $GS^{\bullet}$ signal response being MCFA and FFA, respectively. Furthermore, all fenamates demonstrated MPO-catalyzed cytotoxicity in HL-60 cells, where the most and least toxic compounds were observed to be MCFA and NPA, respectively. Through immunoblotting, we also found that all fenamates induced the formation of protein free radicals in HL-60 cells. **Conclusion:** These findings revealed a correlation between pro-oxidant metabolite reactivity and cytotoxicity caused by fenamates. As such, we discovered that the MPO-mediated oxidation of fenamate NSAIDs lead to the formation of reactive metabolites that cause oxidative damage and induces leukemic cell death.

*O-33 – JUDITH (JUNRAN) YAN

University of British Columbia, Vancouver, BC CANADA

efk-1/eEF2K mediates defense against starvation-induced oxidative stress in C. elegans

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In solid tumors, nutrient deprivation leads to energy imbalance and accumulation of oxidative stress and DNA damage. Over time, cancer cell clones emerge that aberrantly activate a range of stress responses to adapt and survive. Many cancers feature aberrant upregulation of eEF2K, a starvation response regulator that also mediates acute oxidative stress defense. However, it is unknown whether eEF2K promotes starvation survival via increasing oxidative stress resistance downstream. eEF2K is structurally and functionally conserved in C. elegans as efk-1, which is essential for starvation survival. Thus, we exploit the genetic tractability of C. elegans to dissect the efk-1 mediated stress response pathway. First, we asked if efk-1 suppresses ROS generation during starvation, specifically mitochondrial ROS. Indeed, efk-1 mutants accumulate excess ROS during starvation, and antioxidant supplementation is sufficient to rescue their starvation survival defect to WT levels. Supplementation with mitochondrially targeted antioxidant mitoquinone (MitoQ) partially rescued the survival defect of efk-1 mutants, indicating that efk-1 mutants suffer from a detrimental accumulation of ROS produced by the mitochondria. Furthermore, we also observed higher respiration rates in the efk-1 mutant during starvation, which may explain the increased ROS. Next, we asked if efk-1 activates a transcriptomic program for oxidative defense.

We identified two TFs that function in the efk-1 pathway, ZIP-2/bZIP and CEP-1/p53, which upregulate transcription of DNA repair genes in starvation, such as nucleotide excision repair (NER) and base excision repair (BER) genes. Consistently, we confirmed by functional studies that several NER and BER genes are involved in efk-1 mediated starvation resistance using the population starvation survival assay. Notably, exposure to starvation increases resistance to UV-induced DNA damage in wildtype animals, but not the efk-1 mutant. These suggest that efk-1 is required to activate DNA repair during starvation to promote genome integrity. Taken together, we propose a model where efk-1 promotes starvation survival via a translation elongation-independent pathway that involves preserving mitochondrial function, preventing accumulation of mitochondrial ROS and promoting repair of oxidative DNA damage.

*O-34 – BJOERN ZIEHR

Libin Cardiovascular Institute, Cumming School of Medicine, University of Calgary, AB CANADA

IMPACT OF REACTIVE OXYGEN SPECIES ON A CYTOSOLIC PATTERN RECOGNITION RECEPTOR: NUCLEOTIDE-BINDING, LEUCINE RICH REPEAT, PYRIN CONTAINING 3 (NLRP3)

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BACKGROUND: Nucleotide-binding, leucine rich repeat, pyrin containing 3 (NLRP3) is a cytosolic pattern recognition receptor which responds to a diverse set of danger signals. These stimuli induce the ATPase-dependent activation of NLRP3, which then nucleates the assembly of a heterooligomeric inflammasome complex with subsequent production of pro-inflammatory cytokines IL-1B and IL-18. This activation occurs in a two-step model, with a 'priming' signal followed by an 'activation' signal. Many studies highlight the dependency of reactive oxygen species (ROS) for inflammasome assembly, yet none have demonstrated a mechanism. Moreover, cysteines can transduce REDOX signalling in other biological contexts, where oxidation or reduction of critical regulatory cysteines modulates protein activity akin to protein phosphorylation. This study aims to identify whether cysteines of NLRP3 are responsible for ROS sensing and contribute to inflammasome activation.

METHODS: NLRP3-GFP was ectopically expressed in HEK-293T cells and purified using GFP camelid nanobodies. NLRP3 protein was oxidized ($CuCl_2$), blocked (iodoacetamide), reduced (DTT) and labelled (Biotin-maleimide or N-ethylmaleimide). NLRP3 cysteine oxidation was measured broadly by migration impairment during PAGE or specifically by mass spectrometry. Additionally, purified NLRP3 was oxidized and its ATPase activity measured. Moreover, THP-1 derived macrophages were treated with LPS and nigericin to induce inflammasome activation and concomitantly with a cell-permeable probe (1-(pent-4-yn-1-yl)-1H-benzo[c][1,2]thiazin-4(3H)-one 2,2-dioxide, BTD) that is reactive against sulphenes (Cys-SOH), enabling in situ labelling of cysteine oxidation during inflammasome activation.

RESULTS/CONCLUSIONS: Western blot analysis of oxidatively labelled NLRP3 shows that indeed NLRP3 is susceptible to oxidation in vitro. Mass spectrometry analysis identified 15 cysteines that are oxidized, 6 of which are oxidized in controls which suggests these are native disulphide forming cysteines. Additionally, in vitro oxidation of NLRP3 revealed a dramatic (50%) decrease in ATPase activity. Macrophages undergoing inflammasome activation in cellulo displayed a significant increase in total cysteine oxidation compared to macrophages treated with only priming signal. This indicates that REDOX signalling occurs concurrent to the initiation of inflammasome activation. Overall, NLRP3 senses ROS with oxidation of specific cysteine residues. NLRP3 ATPase activity is inhibited by oxidation which supports a model whereby ROS attenuates ATPase activity and maintains NLRP3 in an ATP-bound / 'ON' state.

PLENARY SESSION 6 – OXIDATIVE STRESS RESPONSES IN PLANTS AND MICROORGANISMS

O-35 – SIMON LABBÉ

Université de Sherbrooke, Sherbrooke, QC CANADA

A new role for the peroxiredoxin Tpx1 and sulfiredoxin Srx1 as heme scavengers.

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The model organism Schizosaccharomyces pombe can assimilate heme from external sources in the context where the synthesis of endogenous heme is prevented. S. pombe possesses two systems that are able to function independently in exogenous heme acquisition. One system requires the iron-regulated GPI-anchored protein Shu1 expressed on the cell surface under low-iron conditions. A second heme uptake system relies on the presence of the cell surface transmembrane Str3. Expression of Str3 leads to cellular accumulation of hemin in heme synthesis-deficient hem1Δ cells lacking Shu1. With the goal of further characterizing the Str3-dependent heme acquisition pathway, we found that the peroxiredoxin Tpx1 and sulfiredoxin Srx1 are binding partners of Str3 when hem1Δ cells are exposed to exogenous hemin under low iron conditions. Although Tpx1 is constitutively expressed with no significant iron-dependent changes of its expression profile, we found that its interacting partner Srx1 is transcriptionally repressed by iron in a Fep1-dependent manner. Under microaerobic and low iron conditions, cells that cannot synthesize heme de novo and lack Shu1 exhibit poor hemin-dependent growth in the absence of either Tpx1 or Srx1. Analogous to Tpx1, Srx1 exhibits an enrichment with the membrane fraction containing Str3 in the presence of hemin. Taken together, our results showed that Tpx1 and Srx1 are required for maximal hemin-dependent growth of Str3-expressing hem1Δ shu1Δ cells in ALA-free medium supplemented with hemin under microaerobic conditions.

*O-36 – CHARLIE BOUTIN

Institut de recherche en Biologie végétale, Université de Montréal, Montréal, QC CANADA

In vitro deglutathionylation of a cytosolic aldolase from Arabidopsis thaliana

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In plants, reactive oxygen species (ROS) are by-products of respiration and photosynthesis as well as several other metabolic processes. Biotic or abiotic environmental stresses can disrupt cell redox homeostasis, leading to important increases in ROS levels. During their evolution, plants have developed mechanisms to control ROS levels and minimize oxidative cell damage. Protein thiols present on cysteine residues are sensitive to oxidation by H_2O_2 and can be reversibly modified to generate a sulfenic acid. Further oxidation by H_2O_2 can lead to the irreversible formation of sulfinic and sulfonic acids. One of the mechanisms that evolved to avoid irreversible oxidative damage to proteins is the modification of thiols by glutathione (S-glutathionylation). Several studies have shown an increase in protein S-glutathionylation following oxidative conditions promoted by biotic or abiotic stresses. In addition to its protective effect, S-glutathionylation can affect the activity of its target proteins and, thus, be involved in stress signaling. The reverse reaction, called deglutathionylation, is generally considered to be catalyzed by glutaredoxins (GRX) in plants. Like several enzymes of primary metabolism, cytosolic aldolase has been identified as a target of inhibition by S-glutathionylation in oxidative conditions. However, its deglutathionylation has not been explored. In the present study, we show that aldolase S-glutathionylation can be induced in vitro by diamide and physiological concentrations of reduced glutathione (GSH). We also show that its deglutathionylation can be promoted by incubation with GRXC1, a cytosolic GRX isoform, in the presence of GSH. Surprisingly, we also found that the deglutathionylation of aldolase in vitro is efficiently mediated by physiological concentrations of GSH without GRXC1 catalysis. GSH-mediated deglutathionylation restores aldolase activity and is accompanied by changes in protein migration patterns in polyacrylamide gel electrophoresis. These results constitute a rare example of non-enzymatic deglutathionylation in plants. They also suggest

that changes in the cell redox potential and/or GSH availability could potentially regulate cytosolic aldolase activity in vivo without enzyme involvement.

*O-37 – JASMINE OUELLET

Institut de recherche en biologie végétale, Université de Montréal, Montréal, QC CANADA

The story of Arabidopsis Low expression of Osmotically responsive genes 2: One locus, two proteins and multiple redox modifications

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The Low expression of osmotically responsive genes 2 (LOS2) locus is unusual due to the fact that its sequence encodes two very different proteins due to an alternative translation mechanism. The first gene product is enolase 2 (ENO2), a glycolytic enzyme responsible for most of the cellular phosphoenolpyruvate production. The second protein is LOS2, a transcription factor that participates in the response to cold temperatures, abscisic acid signalisation and osmotic stress. Most of the mechanisms behind the regulation of these two proteins are still poorly understood. In the present work, we demonstrate how ENO2 activity can be regulated by S-glutathionylation, a posttranslational modification that generally plays a protective role against oxidation of cysteine thiols. The reversible formation of a mixed disulfide bridge between ENO2 and glutathione leads to an increase in the catalytic activity of the enzyme. Since all the redox-sensitive cysteines of ENO2 are conserved in the LOS2 sequence, there is a possibility that this transcription factor is regulated by the redox state of the cell as well. Progress towards the study of LOS2 have been hampered by its low yield and solubility. After improving LOS2 purification protocols, we were able to demonstrate the sensibility of LOS2 to oxidative conditions and its modification by S-glutathionylation. Analyses by electrophoresis indicated a modification of molecular mass in response to its oxidation. Mass spectrometry studies were also conducted on ENO2 and LOS2 with the aim of identifying redox-active Cys residues. These analyses have allowed to identify residues involved in redox regulation in ENO2 and LOS2. They also confirm the formation of disulfide bridges involved in the oligomerization of LOS2. The potential significance of our results will be discussed in relation to oxidative stress and the known functions of ENO2 and LOS2 in Arabidopsis.

*O-38 – DILJOT KAUR

Institut de recherche en biologie végétale, Université de Montréal, Montréal, QC CANADA

Pseudophosphorylation of Arabidopsis jasmonate biosynthesis enzyme lipoxygenase 2 via mutation of Ser600 inhibits enzyme activity

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Jasmonates are a class of oxylipin plant hormones crucial for plant defence against chewing herbivory or attack by necrotrophic pathogens. Lipoxygenase2 is an important enzyme involved in an early step of jasmonate biosynthesis. Previous research reported a post-translational modification of arabidopsis Lipoxygenase2 (AtLOX2), where Ser^{600} was constitutively phosphorylated. However, the enzyme was dephosphorylated in mechanically damaged plants. Wildtype recombinant AtLOX2 has positive cooperative behaviour and higher enzyme velocity at high pH with the endogenous substrates a-linolenic acid (a-LeA) and linoleic acid (LA). Using phosphovariants of the Ser^{600}, pseudophosphorylation of Ser^{600} had basal enzyme activity. In contrast, the non-phosphorylatable $Atlox2^{S600A}$ variant had high activity with all the substrates. Multiple sequence alignment of plant and mammal lipoxygenases revealed a conservation of serine or threonine with the arabidopsis Ser^{600} in 45% of plant lipoxygenases associated with defence. Alfa-fold protein modelling of AtLOX2 displayed the critical positioning of the Ser^{600} bordering the catalytic site within the LOX2 structure, highlighting how phosphorylation at this position negatively affects enzyme activity. In conclusion, this study indicates a key mechanism for the regulation of AtLOX2 activity by phosphorylation of Ser^{600}, and hence, the jasmonate biosynthesis.

O-39 – ÉRIC MASSÉ

University of Sherbrooke, Sherbrooke, QC CANADA

Tuning the expression of Fe-S cluster machineries by a regulatory RNA

Karine Prévost[1], Carlos Daniel Vega Valle[1], Marie-Hélène Normand[1], Aura-Lee Béliveau-Caron[1], David Lalaouna[1], Eric Massé[1]

[1]University of Sherbrooke

Iron (Fe) is an essential element for life because it plays a critical role in key biological processes such as respiration, photosynthesis, and DNA synthesis. Additionally, Fe is important for the stability and function of the cell membrane and plays a role in the transportation and metabolism of other nutrients such as nitrogen and sulfur. However, excessive Fe buildup causes toxicity by catalyzing the formation of highly reactive and damaging hydroxyl radical (·OH) through the Fenton reaction. Accumulation of ·OH leads to oxidative stress by causing damages to DNA, RNA, proteins, and lipids. To prevent excess Fe accumulation, most organisms use the formation of Fe-S clusters, which are fundamental and evolutionary ancient cofactors. There are about 140 proteins using Fe-S cluster that can transfer electrons for many biological processes, including respiration, photosynthesis, and DNA repair. Fe-S clusters can undergo rapid and reversible structural conformations to adapt to changes in the local environment, such as variations in pH or redox potential, and to adjust their electron-transfer properties accordingly. There are several pathways for Fe-S cluster biosynthesis, including the ISC\HSC (Fe-S cluster) and SUF (sulfur mobilization) machineries found in bacteria and mitochondria. The expression of ISC\HSC and SUF Fe-S cluster machineries in bacteria has been mostly studied at the promoter level by the Fe-sensing transcriptional factor Fur. We present evidence that both ISC\HSC and SUF operons are regulated post-transcriptionally by a regulatory RNA. The regulatory RNA, RyhB, allows fine regulation of both operons, ensuring expression of ISC\HSC during normal growth condition and SUF during Fe depletion condition.

PLENARY SESSION 7 – NEURODEGENARATIVE DISEASES

O-40 – AYDA TAVITIAN

McGill University, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC CANADA

Heme oxygenase-1 in the Pathogenesis of Schizophrenia

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Schizophrenia is a neuropsychiatric disorder of unknown etiology. Oxidative and other perinatal and early-life stressors have been identified as risk factors for the illness, but it is not known if these stressors are funnelled through select 'transducer' molecules into pathogenesis of the disorder. Heme oxygenase-1 (HO-1) is a protein that is readily upregulated in cells in response to numerous oxidative and inflammatory stressors known to increase risk for developing schizophrenia. Oxidative stress is elevated in the brains of GFAP.HMOX1$^{(0\text{-}12m)}$ transgenic mice that overexpress HO-1 in astrocytes from embryogenesis to adulthood. We determined the behavioural, neuroanatomical, neurochemical and gene expression profiles of these mice in early and mid-adulthood and found that adult GFAP.HMOX1$^{(0\text{-}12m)}$ transgenic mice exhibit many schizophrenia-relevant features. Behavioural anomalies include hyperkinetic behaviour, increased motor stereotypy, impaired nest-building capacity mirroring dysfunction in self-care and activities of daily living in humans, reduced preference for social novelty, impaired working memory and deficient prepulse inhibition of the acoustic startle response. Neuroanatomical defects are evident as ventriculomegaly and dysgenesis of the hippocampal dentate gyrus and of the corpus callosum. Neurochemical perturbations include augmented dopamine and serotonin levels in basal ganglia. The expression of reelin is reduced in several brain regions of male mice. Hyperkinesia and motor stereotypy in GFAP.HMOX1$^{(0\text{-}12m)}$ mice were attenuated by both clozapine, an atypical antipsychotic medication, and Immunocal, a glutathione precursor nutraceutical. Immunocal also augmented brain reelin content but did not correct established anatomical defects of the brain. The GFAP.HMOX1$^{(0\text{-}12m)}$ neurophenotype implicates astrocytic HO-1 as a transducer of early-life stressors into schizophrenia-related pathophysiology. Containment of the glial HO-1 response to such stressors may provide novel preventive or therapeutic strategies for neurodevelopmental disorders like schizophrenia.

O-41 – CHARLES RAMASSAMY

INRS-Centre Armand Frappier-Santé Biotechnologie, Laval, QC CANADA

Dual role of circulating exosomes in Alzheimer's disease: the vehicle for redox, inflammatory markers and the amyloid-peptide propagation or clearance?

Mohamed Haddad[1], Mohamed Raâfet Ben Khedher[1], Hermine Counil[1], Rummenigge Oliveira Silva[1], Charles Ramassamy[1]

[1]INRS

Alzheimer's disease (AD) is an age-related brain disorder and the leading cause of dementia. Oxidative stress is a unifying paradigm in the pathophysiology of AD. The oligomeric amyloid-b peptide (oAb) and presence of the apolipoprotein E4 variant (APOE ε4) are known to stimulate oxidative damage and enhance AD risk.

Exosomes or extracellular vesicles (EVs) (50-150 nm) are released by all cell types in the body.

The objective of our study was to investigate the role of cEVs in the propagation of redox, inflammatory and the oAb in the brain. For this, we have determined the impact of APOE ε4 on the level of apolipoproteins with antioxidant activities (apoE, apoJ, and apoD) along with oxidative markers in circulating extracellular vesicles (cEVs) and plasma from cognitively impaired-not demented (CIND) individuals converted to AD (CIND-AD) and a panel of inflammatory markers in cEVs and plasma from AD patients. Finally, we have investigated the role of cEVs in the clearance of oAb.

Methods: EVs were isolated using the Total Exosome Isolation reagent and characterized according to the ISEV guidelines. Apolipoproteins with antioxidant activities (E, J, D), antioxidant response and inflammatory markers were determined in cEVs and plasma using immunoblotting, electrochemical examination, spectrofluorimetry and ELISA.

Results: We observed a significant decrease in the total antioxidant capacity (TAC) in the CIND-AD group. Levels of apoD in plasma and cEVs were higher in CIND-AD participants. Interestingly, protein carbonyls content and apoJ/D ratio were statistically different in cEVs but not in plasma from CIND-AD. Our data also indicate that TAC, cEVs protein carbonyls, cEVs apoJ/D levels were correlated with the neurocognitive Mini-Mental State Exam (MMSE) scores and are APOE ε4-dependant. Some inflammatory markers in cEVs were higher than in plasma. We found that oAβ were topologically bound to the external surface of cEVs.

Conclusion: Our findings support the pathological redox linkage between APOE ε4 and AD onset and suggest the use of cEVs oxidative signature and some inflammatory markers in early AD diagnosis. Our results also support that cEVs might participate in oAβ clearance.

Supports: Research Chair Louise & André Charron on Alzheimer's disease, the Armand-Frappier Foundation, MRIF and INAF.

O-42 – EFTEKHAR EFTEKHARPOUR

University of Manitoba, Winnipeg, MB CANADA

Neuronal Thioredoxin-1 depletion: from Cellular Deficits to widespread CNS degeneration.

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Thioredoxin-1 (Trx1) is a major oxidoreductase cytoplasmic protein involved in regulation of peroxides activity and regeneration of oxidized proteins. An expanding body of literature has linked decreased levels of Trx1 activity to neurodegenerative diseases. Although the underlying mechanisms of Trx1 depletion are not fully examined, we asked whether depletion of Trx1 is sufficient to induce neurodegeneration.

We have detected evidence of Trx1 depletion in human post-mortem brain tissue from AD patients and 3XTg mice model of AD. Using neuroblastoma SH-SY5Y, primary neuronal cultures we show that pharmacologic and genetic inhibition of Trx1 results in robust cytoplasmic and nuclear changes, including decreased autophagy and lysosomal activity, cytoskeletal reorganization and increased actin fibrilization as well as nuclear lamina invagination and chromatin reorganization. Emerging reports have uncovered evidence of neuronal nuclei involvement in pathophysiology of AD.

Using a mouse model of neuronal Trx-1 knockout, we observed significant structural and functional deficits in central nervous system as shown by increased neuronal degeneration, decreased myelination and enhanced glial reaction. Motor deficiency in these animals was associated with degeneration of spinal neurons and disrupted cytoplasmic-nuclear transport and cytoplasmic accumulation of TDP-43 and Nrf2. Additionally, these animals show evidence of ataxia and die suddenly with evidence of epileptic seizure. These data further indicate the protective role of Trx1 and its importance in pathophysiology of neurodegenerative diseases.

PLENARY SESSION 8 – OXIDATIVE STRESS IN AGING AND MITOCHONDRIAL DISEASE

O-43 – CHRISTOPHER PERRY

York University, Toronto, ON CANADA

Targeting mitochondrial cardiolipin to preserve bioenergetics and muscle health in Duchenne muscular dystrophy

Christopher Perry[1], Meghan Hughes[1], Sofhia Ramos[1], Catherine Bellissimo[1], Athan Dial[2], Thomas Hawke[2]

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Duchenne muscular dystrophy (DMD) is a genetic-based disease which causes severe muscle wasting and weakness, resulting in loss of ambulation and premature death due to cardiac and/or respiratory failure. Current standard of care focuses on treating secondary contributors to the DMD myopathy and as such, there is a clear need to develop a portfolio of potential therapeutic candidates that target the underlying causes of muscle degeneration in this disease. Here we evaluate the efficacy of the mitochondrial -targeted peptide SBT-20 in improving DMD pathophysiology through the amelioration of impairments in mitochondrial bioenergetics. Beginning at 4 days of age, D2.B10-DMDmdx/2J (D2.mdx) mice received daily injections of 5mg/kg SBT-20 or volume equivalent saline for 28 days. A wildtype group was also evaluated at this time point. Following treatment, mitochondrial bioenergetics (oxidative phosphorylation, mitochondrial H_2O_2 emission) were partially preserved in diaphragm and quadriceps muscle. These preservations were associated with elevated force production in the diaphragm and decreased fibrosis in the quadriceps. The ultra-structure of quadriceps mitochondria was also preserved following treatment, despite a surprising increase in susceptibility to calcium-induced mitochondrial permeability transition pore opening and mitochondrial-derived caspase activity. Furthermore, while body weight was unchanged, lower limb muscle volume improved, as did forelimb grip strength. These improvements were evident at a very early stage in disease progression. Collectively, these findings demonstrate that SBT-20 improved specific bioenergetic and muscle quality indices and demonstrates that pharmacological targeting of mitochondria could be a potential avenue for therapy development in Duchenne muscular dystrophy.

O-44 – SIEGFRIED HEKIMI

McGill University, Montreal, QC CANADA

Converting coenzyme Q from inefficient nutraceutical to an actual treatment option for coenzyme Q deficiency and other diseases

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Coenzyme Q_{10} (CoQ_{10}; also known as ubiquinone) is a crucial, redox-active membrane component that functions as obligate electron transporter in the mitochondrial respiratory chain, as cofactor in other enzymatic processes and potentially as antioxidant. CoQ_{10} supplementation has been widely investigated for treating a variety of acute and chronic conditions in which mitochondrial function or oxidative stress play a role. In addition, it is used as replacement therapy in patients with CoQ deficiency including inborn primary CoQ_{10} deficiency due to mutations in CoQ_{10}-biosynthetic genes as well as secondary CoQ_{10} deficiency, which is frequently observed in patients with mitochondrial disease syndrome and in other conditions. However, despite many tests it remains doubtful whether oral CoQ_{10} treatment can be beneficial in any indication. In fact, our recent review of the outcomes of CoQ_{10} treatments of patients with primary CoQ_{10} deficiency suggests that there are no, or only very minimal, effects of treatment. Because CoQ_{10} is highly insoluble, it is only available in oral formulations. Using a novel model of CoQ-deficient cells, we screened a library of FDA-approved drugs for an activity that could increase the uptake of exogenous CoQ_{10} by cells. We identified the FDA-approved intravenous fungicide caspofungin as capable of increasing the aqueous solubility of CoQ_{10} by several orders of magnitude. Caspofungin is a mild surfactant that solubilizes CoQ_{10} by forming nano-micelles with unique properties favoring stability and cellular uptake. Intravenous administration of the formulation in mice achieves unprecedented increases in CoQ_{10} plasma levels and in tissue uptake, with no observable toxicity. As it contains only two safe components (caspofungin and CoQ_{10}), this injectable formulation presents a high potential for clinical safety and efficacy.

A complete deficiency in CoQ_{10} synthesis is lethal. All patients presenting with CoQ_{10} deficiency therefore still synthesis CoQ_{10}, albeit at an insufficient level. Our new formulation using caspofungin for intravenous administration seeks to supplement the deficiencies by supplying exogenous CoQ_{10}. As a complementary approach we are seeking to identify drugs capable of boosting endogenous CoQ_{10} synthesis. Such drugs could be applied to cases of CoQ_{10} deficiency but also to boost CoQ_{10} in diseases with features of mitochondrial dysfunction and/or oxidative stress, including neurodegenerative diseases. Progress in the identification of such drugs will be presented at the meeting.

O-45 – RYAN J. MAILLOUX

McGill University, Ste-Anne-de-Bellevue, QC CANADA

Not just powerhouses anymore: mitochondria are central hubs for cell redox signaling too.

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Peter Siekevitz called the mitochondrion the “powerhouse of the cell” for good reason. These double membrane organelles specialize in energy conservation by generating a protonmotive force from the transfer of electrons transfer from nutrients to molecular oxygen, a process driven by flavin-dependent dehydrogenases and high molecular weight membrane bound respiratory complexes (REF). The free energy stored in this transmembrane potential of protons is used by complex V to produce ATP. The electron transfer reactions required to establish a protonmotive force also generate hydrogen peroxide (H_2O_2), a “mitokine” used in cell signaling. Mitochondria use several mechanisms to control H_2O_2 production for cell signaling. These mechanisms are also used to prevent H_2O_2 cytotoxicity since higher levels can overwhelm antioxidant defenses and cause oxidative distress. Our group has been focused on understanding the feedback mechanisms used by mitochondria to control the production of H_2O_2 for cell signaling and the prevention of oxidative distress. Mechanisms used to feedback and inhibit H_2O_2 genesis by flavin-dependent dehydrogenases and respiratory complexes I, II, and III include proton leaks and the redox modification of protein thiols. The former mechanism decreases H_2O_2 production by lowering protonic backpressure on the respiratory chain, limiting electron accumulation and the over-reduction redox centers in the chain that generate reactive oxygen species (ROS). The second mechanism involves the covalent modification of proteinaceous thiols through redox reactions. Here, I will discuss findings demonstrating redox signals like protein S-glutathionylation, which involves the addition and removal of the tripeptide antioxidant glutathione (GSH) to and from cysteine, serves as a negative feedback loop for the inhibition of H_2O_2 production by several dehydrogenases including α-ketoglutarate dehydrogenase (KGDH), pyruvate dehydrogenase (PDH), complex I, and dihydroorotate dehydrogenase (DHODH). I will discuss the importance of this feedback loop in the desensitization of mitochondria-to-cell H_2O_2 signals in health and disease and our discovery of a sex dimorphism in the mitochondrial glutathionylation pathway. Finally, I will also discuss emerging evidence from our group demonstrating S-nitrosylation reactions may fulfill a similar role and that adding nitro groups to cysteines may also be subjected to sex regulation.

O-46 – JEREMY VAN RAAMSDONK

McGill University, Montreal, QC CANADA

The complex relationship between oxidative stress and aging: How a mild increase in mitochondrial ROS can extend longevity

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Oxidative stress increases with advancing age and is associated with the development of age onset neurodegenerative disease. On the other hand, a mild increase of reactive oxygen species (ROS) can result in lifespan extension. Mutations in that cause a small impairment of mitochondrial function increase lifespan and enhance biological resilience despite increasing levels of ROS. In fact, the increase in ROS contributes to the extended longevity as treatment with antioxidants reduces lifespan towards wild-type. We have examined gene expression changes in long-lived mitochondrial mutants using RNA-sequencing as an unbiased approach to gain insight into the molecular mechanisms contributing to lifespan extension. Our results indicate that multiple pathways of cellular resilience are activated in the long-lived mitochondrial mutants and contribute to their longevity. These pathways include the DAF-16 mediated stress response, the mitochondrial unfolded protein response, the p38-mediated innate immune signaling pathway and the mitochondrial thioredoxin system. Overall, our research demonstrates that small amounts of oxidative stress can increase lifespan and resistance to stress through the activation of stress response pathways.

CONFÉRENCIER D'HONEUR / KEYNOTE LECTURE 3

O-47 – KEYNOTE SPEAKER: SHARON CAMPBELL

Lineberger Cancer Center at the University of North Carolina, Chapel Hill, NC USA

Redox Regulation of RAS and RHO GTPases

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The recent development of mutant-selective inhibitors for the oncogenic $KRAS^{G12C}$ allele has generated considerable excitement. These inhibitors covalently engage the mutant C12 thiol located within the phosphoryl binding loop of RAS, locking the $KRAS^{G12C}$ protein in an inactive state. While clinical trials of these inhibitors have been promising, mechanistic questions regarding the reactivity of this thiol remain. Here, we show by NMR and an independent biochemical assay that the pK_a of the C12 thiol is depressed (pK_a ~7.6), consistent with susceptibility to chemical ligation. Using a validated fluorescent $KRAS^{Y137W}$ variant amenable to stopped-flow spectroscopy, we characterized the kinetics of $KRAS^{G12C}$ fluorescence changes upon addition of ARS-853 or AMG 510, noting that at low temperatures, ARS-853 addition elicited both a rapid first phase of fluorescence change (attributed to binding, K_d = 36.0 ± 0.7 μM) and a second, slower pH-dependent phase, taken to represent covalent ligation. Consistent with the lower pK_a of the C12 thiol, we found that reversible and irreversible oxidation of $KRAS^{G12C}$ occurred readily both in vitro and in the cellular environment, preventing the covalent binding of ARS-853. Moreover, we found that oxidation of the $KRAS^{G12C}$ Cys12 to a sulfinate altered RAS conformation and dynamics to be more similar to $KRAS^{G12D}$ in comparison to the unmodified protein, as assessed by molecular dynamics simulations. Taken together, these findings provide insight for future $KRAS^{G12C}$ drug discovery efforts, and identify the occurrence of G12C oxidation with currently unknown biological ramifications.

O-48 – VOLKER BLANK

Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University, Montreal, Qc CANADA

Linking CNC transcription factor function to stress signaling and inflammation

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Colorectal cancer (CRC) is the second most deadly cancer in Canada. Patients with inflammatory bowel disease (IBD) are at increased risk of developing CRC. Both, CRC and IBD have been associated with inflammation and oxidative stress. Recent studies showed that in contrast to late onset CRC (>50y), whose numbers have been decreasing slightly, early onset CRC (18-49y) is on the rise worldwide. Early onset CRC has been linked to IBD, is confined primarily to the left colon or rectum and is often detected only at advanced tumor stages.

In earlier studies, we have shown that knockdown of the CNC transcription factor NFE2L3 leads to a significant decrease of human CRC cell growth. We investigated the role of NFE2L3 using the AOM/DSS mouse model of inflammation induced colorectal cancer. Nfe2l3$^{-/-}$ mice displayed less inflammation and a reduced number of tumors. We showed that Nfe2l3 transcript levels are increased in the distal portion of wild-type mice. KEGG pathway enrichment analysis of oxidative stress markers revealed elevated NOX1 and NQO1 levels in Nfe2l3$^{-/-}$ mice and increased HO1 expression in wild-type mice in AOM/DSS induced CRC tumors. With respect to immune cells, we observed a reduction of mast cells and an increase in immunosuppressive Tregs in the colon of Nfe2l3$^{-/-}$ animals. Additional experiments inducing colitis with treatment of DSS alone, revealed that expression of the Lipocalin-2 (LCN2) gene, coding for an inflammatory mediator, was increased in all colon sections after DSS treatment except in the distal (left) colon of Nfe2l3$^{-/-}$ mice. LCN2 protein levels in the stool were also augmented in wild-type and Nfe2l3$^{-/-}$ mice compared to non-inflamed mice, with a less important increase in the knockout animals. Database analyses in humans showed increased levels of the transcription factor in the rectum (left side) in ulcerative colitis patients. Our data revealed that NFE2L3 promotes CRC in the context of an underlying inflammatory condition by modulation of the microenvironment. As sidedness, the location of the tumor within the colon, has become an important prognostic and predictive factor, our results may be particularly relevant for early onset CRC, which is often found in the left colon.

O-49 – ALAIN NEPVEU

Rosalind and Morris Goodman Cancer Institute, McGill University, Montreal, QC CANADA

Oxidative DNA Damage, Transcriptional Regulation, Senescence and Cancer Cell Adaptation

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Endogenously produced reactive oxygen species (ROS) are an important source of oxidative DNA damage, particularly in some cancer cells. Recent studies in this field revealed multiple connections between oxidative DNA damage repair and transcription factors. On the one hand, some enzymes of the base excision repair (BER) pathway were found to recruit transcription factors to the promoters of specific genes leading to their activation. On the other hand, some transcription factors were found to function as accessory factors that stimulate the enzymatic activities of BER enzymes and accelerate repair of oxidative DNA lesions. Strikingly, some of these accessory factors are overexpressed in cancer cells. Altered metabolism in cancer cells leads to increased ROS production that causes oxidative DNA damage and ultimately, cellular senescence. In this context, senescence functions as a tumor suppressor mechanism. Unfortunately, some rare cells can adapt to become cancerous. One well described mechanism of adaptation involves increased expression of antioxidants to re-establish ROS homeostasis. We have uncovered a second mechanism of adaptation whereby cancer cells elevate expression of BER enzymes and accessory factors (CUX1, CUX2, STAB1, BCL11A, BCL11B) to increase their capacity to repair oxidative DNA damage, avoid senescence and continue to proliferate. Paradoxically, two genes encoding oncogenic accessory factors, CUX1 and BCL11B, were also characterized genetically as tumor suppressor genes. We find that TK6 cells in which one allele of CUX1 or BCL11B has been inactivated exhibit higher spontaneous and radiation-induced mutation rates, a phenotype that is predicted to increase the probability of developing cancer.

O-50 – JOSIE URSINI-SIEGEL

Lady Davis Institute for Medical Research, McGill University, Montréal, QC CANADA

p66ShcA-induced metabolic reprogramming maintains redox balance to promote the emergence of aggressive breast cancers.

Josie Ursini-Siegel[1]
[1]Lady Davis Institute for Medical Research

Aggressive breast cancers, irrespective of their molecular subtype, must capitalize on two key biological processes to grow, survive and metastasize. These include: (1) metabolic reprogramming to increase their metabolic rate and acquire the ability to consume multiple metabolites to meet their energetic and biosynthetic demands and (2) redox balance to capitalize on the tumor-promoting properties of reactive oxygen species (ROS), while preventing deleterious oxidative damage to protein, lipids and DNA. Of relevance to this project, the ShcA gene encodes three isoforms from two distinct promoters. The two shorter isoforms (p46/52) are ubiquitously expressed and transduce mitogenic signals that are essential for breast cancer initiation and progression. In contrast, p66ShcA is variably expressed in breast cancers and is transcribed from a unique promoter. At steady state p66ShcA resides in the cytoplasm and functions as an adaptor protein to engage distinct signaling pathways. However, in response to a variety of stress stimuli, p66ShcA becomes phosphorylated, which induces its translocation into the mitochondrial inner membrane space where it stimulates ROS production. The goal of this study seeks to elucidate how p66ShcA-expressing breast tumors evolve towards a path of increased aggressiveness in the face of moderately elevated and sustained oxidative stress. In independent pre-clinical models, we demonstrate that p66ShcA sculpts the evolution of such aggressive breast tumors by inducing metabolic reprogramming to favor catabolic metabolism to preserve cellular ATP levels under stressed conditions, including nutrient deprivation and anchorage independent growth. In addition, p66ShcA paradoxically is required to maintain redox balance by increasing glutathione production and utilization in cancer cells under such stressed environments. By doing so, chronic exposure to p66ShcA increases the metabolic plasticity of breast cancer cells to potentiate their metabolic potential. Finally, we also show that this p66ShcA is also associated with improved redox balance in primary breast cancers.

O-51 – VIVEK VENKATARAMANI

University Medicine Göttingen, Robert-Koch-Strasse Göttingen, Germany

APP- and BACH1-Dependent Pathways: New Avenues for Modulating Ferroptosis in Cancer and Neuronal Ischemic Injury

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b-amyloid precursor protein (APP) is widely known as precursor of neurotoxic Ab peptide in Alzheimer's disease. However, the physiological function of the full-length protein remains debatable. Herein, we show that oncogene-mediated malignant transformation induces APP biosynthesis. This confers an adaptive survival advantage for cancer cells by preventing ferroptosis. Consequently, APP is commonly overexpressed in various aggressive tumor types and correlates with poor prognosis. Mechanistically, loss-of-APP results in mitochondrial translocation and enzymatic activation of its interacting partner heme oxygenase-1 (HO-1). Unrestricted heme degradation by HO-1 allows accumulation of heme-regulated transcription factor BACH1. We demonstrate that BACH1 promotes ferroptosis by suppressing responses to oxidative stress, while non-canonically enforcing glycolytic energy supply resulting in polyunsaturated fatty acids (PUFA) synthesis. This mechanism suppresses tumor growth and metastatic colonization but aggravates ischemic injury. Remarkably, pharmacological targeting of BACH1 potentiated neuronal ferroptosis inhibition by liproxstatin-1 and improved behavior in an ischemic stroke model. Thus, APP- and BACH1-dependent pathways offer new potential therapeutic targets to modulate ferroptosis in cancer and neuronal ischemic injury.

PLENARY SESSION 10 – OXIDATIVE STRESS IN CANCER IN HEALTH AND NUTRITION

O-52 – STAN KUBOW

McGill University, Montreal, QC CANADA

Probiotics, the microbiome and the host gut immune system involving Clostridioides difficile infection

Stan Kubow[1]
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Increased abundance of pathogenic gut bacteria such as Clostridioides (C.) difficile (CDI) in proinflammatory disease states is linked with increased oxidative stress and an altered redox balance in the gastrointestinal tract. Pro-oxidative gut pathogens release reactive oxygen species (ROS) via discharge of proinflammatory toxins such as toxins A and B from C. difficile. Probiotic bacteria can maintain intestinal homeostasis by modulation of gut microbiota, its function, host immune response and induction of cellular antioxidant signaling pathways such Nrf2-Keap1-ARE. Probiotics can directly scavenge ROS, act as strong chelators of free copper or iron ions to prevent metal ion-catalyzed oxidation, and release glutathione and antioxidant enzymes. In the present study, normal and CDI-infected fecal samples were treated with several single strain and multispecies probiotics in a simulated gastrointestinal (GI) model. The GI-derived fecal water (FW) was assessed for microbiota composition and function, antioxidant status of the colonic milieu, and its effect on the inflammatory response of T84 human colon epithelial cells. The CDI fecal samples altered the colonic antioxidant status, decreased microbial alpha diversity and decreased short-chain fatty acid (SCFA) production. Probiotic supplementation in the CDI samples showed improved antioxidant status attributed to increased copper chelation. Probiotic supplements restored microbial metabolic function in CDI samples via increased production of SCFAs. T84 cells exposed to CDI-FW exhibited increased cytotoxicity and proinflammatory cytokine production characterized by interleukin (IL)-8, CXCL-5, MIF, TNFRSF8, and IL-32. These effects were diminished with exposure of T84 cells to CDI-FW treated with certain probiotics, such as the single-strain Saccharomyces boulardii CNCM I-1079 and Lactobacillus rhamnosus R0011. Overall, these findings indicate the potential of probiotics to restore intestinal homeostasis in CDI as mediated via improvements in gut microbiota and host intestinal cell functionality.

O-53 – GIADA SEBASTIANI

McGill University Health Centre, McGill University, Montreal, QC CANADA

Oxidative stress in nonalcoholic fatty liver disease

Giada Sebastiani[1]
[1]McGill University Health Centre

Nonalcoholic fatty liver disease (NAFLD) is the most frequent liver disease worldwide and a leading cause of liver transplantation. NAFLD is defined by the accumulation of fat in more than 5% of hepatocytes, detected either by imaging or histology, with no evidence of other causes of underlying liver diseases such as alcohol abuse, viral hepatitis or genetic disorders. The spectrum of this condition is wide, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), where inflammation and hepatocyte injury coexist, possibly evolving to liver fibrosis, cirrhosis and hepatocellular carcinoma. NAFLD affects approximately 25-30% of the global population, with a much higher prevalence in high-risk groups such as patients with type 2 diabetes mellitus or obesity, where it rises up to 60% and 90%, respectively. The pathogenesis of NAFLD is multifactorial, where genetic and environmental factors contribute to the accumulation of fat in the liver and to liver fibrosis progression. Insulin resistance is the recognized key player in the complex NAFLD pathogenesis, however oxidative stress also exerts a major role. Different reactive oxygen species (ROS) generators contribute to NAFLD inflammatory and fibrotic progression, which is linked to the lipotoxic liver injury from fatty acids and/or a wide variety of their biologically active metabolites in the context of a multiple parallel hits theory. For example, iron may contribute to the development or exacerbation of insulin resistance. Indeed, elevation of serum iron indices is frequent in patients with NAFLD and it is an independent predictor of liver fibrosis. Moreover, iron could synergize with lipid overload and induction of cytochrome P450 to increase oxidative stress in hepatocytes. Healthy diet and physical activity have been shown to be effective on NAFLD also with antioxidant mechanisms, but compliance to these lifestyles is suboptimal. Among several antioxidants, vitamin E has been proposed and it is still the first line pharmacotherapy for NASH in absence of approved NASH-targeted treatments. Studies with natural polyphenols proposed for NAFLD prevention and treatment are promising. Probiotics, prebiotics, diet, or fecal microbiota transplantation represent new therapeutic approaches targeting the gut microbiota dysbiosis. Future studies should focus on precision medicine taking into consideration both genetic and environmental epigenetic risk factors for individualized treatment.

O-54 – KIMBERLY DUNHAM-SNARY

Queen's University, Kingston, ON CANADA

Neutrophil Mitochondria Mediate Killing of Staphylococcus Aureus ex vivo

Kimberly Dunham-Snary[1], Bas Surewaard[2], Jeffrey Mewburn[1], Paul Kubes[2], Stephen Archer[1]
[1]Queen's University, [2]University of Calgary

Background: Neutrophils play a role in innate immunity and are critical for clearance of Staphylococcus aureus. Current understanding of neutrophil bactericidal effects is that NADPH oxidase produces reactive oxygen species (ROS), mediating bacterial killing. Neutrophils also contain numerous mitochondria; since these organelles lack oxidative metabolism, their function is unclear. We hypothesize that mitochondria in human neutrophils contribute to the bactericidal capacity of S. aureus.
Methods and Findings: Using human neutrophils isolated from healthy volunteers (n = 13; 7 females, 6 males), we show that mitochondria are critical in the immune response to S. aureus. Using live-cell and fixed confocal, and transmission electron microscopy, we show mitochondrial tagging of bacteria prior to ingestion and surrounding of phagocytosed bacteria immediately upon engulfment. Further, we demonstrate that mitochondria are ejected from intact neutrophils and engage bacteria during vital NETosis. Inhibition of the mitochondrial electron transport chain at Complex III, but not Complex I, attenuates S. aureus killing by 50 ± 7%, comparable to the NADPH oxidase inhibitor apocynin. Similarly, mitochondrial ROS scavenging using MitoTEMPO attenuates bacterial killing 112 ± 60% versus vehicle control. Antimycin A treatment also reduces mitochondrial ROS production by 50 ± 12% and NETosis by 53 ± 5%.
Conclusions: We identify a previously unrecognized role for mitochondria in human neutrophils in the killing of S. aureus. Inhibition of electron transport chain Complex III significantly impairs antimicrobial activity. This is the first demonstration that vital NETosis, an early event in the antimicrobial response, occurring within 5 min of bacterial exposure, depends on the function of mitochondrial Complex III. Mitochondria join NADPH oxidase as bactericidal ROS generators that mediate the bactericidal activities of human neutrophils.

O-55 – NATHALIE GRANDVAUX

CRCHUM, Université de Montréal, Montréal, QC CANADA

Redox sensitive signaling adaptors control the efficiency of the antiviral response.

Nathalie Grandvaux[1, 2]
[1]CRCHUM, [2]Université de Montréal

The antiviral response elicited upon virus sensing by pathogen recognition receptors is mediated by multiple signaling cascades subjected to highly stringent regulatory mechanisms. Post-translational modifications (PTMs) of antiviral signaling proteins, including phosphorylation or ubiquitination, have proven to be key determinants of the intensity and duration of the response. Recently our laboratory and others unveiled that NADPH oxidase-derived reactive oxygen species (ROS) are essential for the induction of antiviral and proinflammatory genes. The molecular mechanisms of action of ROS remained elusive. Redox PTMs (ox-PTMs), notably on Cys residues, are key processes to regulate signaling proteins structure and function. Through a proteome wide redoxome analysis, we identified thousands of Cys residues subjected to ox-PTMs, including some in key adaptors of the antiviral signaling cascades. Thorough molecular biology analyses, we investigated the complex role of these modifications. These studies provide a new level of understanding of the molecular mechanisms that explain the redox regulation of the antiviral response.

O-56 – PREMKUMARI KUMARATHASAN

CRCHUM, Université de Montréal, Montréal, QC CANADA

Air pollution Exposure-Related Adverse Health Effects: Role of Oxidative stress

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Elevated levels of air pollution are associated with increased cardio-respiratory mortality and morbidity. Emerging epidemiological evidence also suggest links between air pollution exposures and neurorological disorders (e.g., Alzheimers, Parkinson), gastrointestinal disorders (e.g., IBD) and adverse pregnancy outcomes (e.g., low birth weight) among others. Air pollution matrix consists of gaseous (e.g., ozone, sulfur dioxide and oxides of nitrogen) fraction and particulate matter (PM). Ambient air PM is composed of different size modes (PM10, PM2.5 and UFP). Systematic toxicology studies are required to gain clarity on air pollutant exposure- driven toxicity pathways. We have conducted in vitro cell culture, in vivo animal exposure (nose-only exposure) studies and human exposure studies in the interest of understanding air pollution exposure-related toxicity pathways. Our in vitro study findings showed PM exposure-related oxidative stress and associated adverse cellular effects (e.g., cell death, inflammatory response). Also, in vivo animal exposure experiments identified air pollutant-specific oxidative stress mechanisms and downstream vascular effects (e.g., endothelinergic system changes) as related to cardiovascular effects. Similarly, we observed air pollutant exposure-related changes in oxidative stress, vascular, and inflammatory markers in healthy humans. We also observed that some of these effects can be modified by intervention strategies (e.g., antioxidant mimic). In addition, our results also showed that PM size and composition can act as key potency determinants. Studies are underway to gain insight into toxicity pathways in air pollution exposure-related adverse pregnancy outcomes to explore causal links in vulnerable populations (mother-infant pair).

O-57 – GONZALO COSA

McGill University, Montreal, QC CANADA

Visualizing lipid peroxidation and electrophilic stress in cells

Gonzalo Cosa[1]
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In this presentation I will describe fluorogenic (off to on) probes we have developed to monitor electron transport, lipid peroxidation[1] and electrophilic stress[2] in lipid membranes, focusing on their recent application to study ferroptosis. Firstly, establishing where and when lipid peroxidation during ferroptosis takes place in a live cell, and monitoring the process real-time, we posit, would facilitate a mechanistic understanding. I will describe live cell imaging work where we exploit newly developed activatable fluorogenic antioxidants[1] and state-of-the-art imaging methodologies to monitor lipid peroxyl radicals. Secondly, I will touch upon the ability of cells to detoxify increasing lipid derived electrophile (LDE) levels during ferroptosis, exploring the link between lipid hydroperoxide accumulation, LDE formation and cell death. Here, I will describe a recently developed assay (ElectrophileQ) that enables live-cell assessment of the glutathione-mediated LDE conjugation and adduct export steps of the LDE detoxification pathway. This method revealed that during ferroptosis, LDE detoxification failure occurs through decreased conjugation or export impairment, amplifying cellular electrophile accumulation.

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LIST OF POSTERS by Primary Authors – 1 to 49

POSTER 1 – Role of bioactive compounds on sodium iodate induced retinal pigment cell damage
Nicholas Bel[1], Sanjoy Gupta[2], Neelam Khaper[1, 2]
[1]Lakehead University, Thunder Bay, ON, [2]Northern Ontario School of Medicine University, Thunder Bay, ON

POSTER 2 – Stimulation of RAS-dependent ROS signaling extends longevity by modulating a developmental program of global gene expression
Robyn Branicky[1], Ying Wang[1], Arman Khaki[1], Maximilian Kramer-Drauberg[1], Siegfried Hekimi[1]
[1]McGill University

POSTER 3 – TXNIP promotes ferroptosis through NCOA4 mediated ferritinophagy
Pandian Nagakannan[1], Shakila Sultana[1], Md Imamul Islam[1], Eftekhar Eftekharpour[1]
[1]Dept. Physiology and Pathophysiology, University of Manitoba

POSTER 4 – Characterizing partners of the C. elegans Nuclear Hormone Receptor NHR-49 in oxidative stress response
Glafira Ermakova[1], Kelsie Doering[1], Stefan Taubert[1]
[1]Centre for Molecular Medicine and Therapeutics, Department of Medical Genetics, University of British Columbia; BC Children's Hospital Research Institute

POSTER 5 – Beta-caryophyllene effects in pulmonary oxidative stress in pulmonary hypertensive rats
Cristina Campos Carraro[1], Patrick Turck[1], Alan Bahr[1], Rafael Oliveira Fernandes[1], Letícia Koester[1], Adriane Belló-Klein[1]
[1]Universidade Federal do Rio Grande do Sul

POSTER 6 – Hepcidin inhibits iron efflux from duodenal ferroportin and thereby indirectly promotes iron-dependent DMT1 degradation
Angeliki Katsarou[1], Carine Fillebeen[1], Kostas Pantopoulos[1]
[1]Lady Davis Institute for Medical Research, Jewish General Hospital, and Department of Medicine, McGill University, Montreal, Quebec, Canada

POSTER 7 – Real-time monitoring of antibiotic-triggered lipid peroxidation in bacterial membranes
Florencia Fungo[1], Sol Martinez[1], Gonzalo Cosa[1]
[1]McGill University

POSTER 8 – Exploring the effects of an experimental autoimmune myositis rodent model on mitochondrial H2O2 emission in skeletal muscle
Madison C. Garibotti[1], Arshdeep K. Thuhan[1], Luca J. Delfinis[1], Shahrzad Khajehzadehshoushtar[1], Ali A. Abdul-Sater[1], Christopher G. R. Perry[1]
[1]School of Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, ON

POSTER 9 – Untargeted and targeted metabolomics by LC-MS/MS for studying metabolic perturbations in biological samples
Nathan Ghafari[1], Lekha Sleno[1]
[1]University of Quebec in Montreal (UQAM), Chemistry Department, Montreal, QC, Canada

POSTER 10 – Examining the effects of 8-oxoguanine on gene expression and mitochondrial biology
Ryan Gillett[1], Alexandria Kellington[1], James Uniacke[1]
[1]University of Guelph

POSTER 11 – An investigation into the effect of sex on the hydrogen peroxide (H2O2) generating capacities of individual sites of production in mouse liver mitochondria
Cathryn Grayson[1], Olivia Koufos[1], Ryan Mailloux[1]
[1]McGill University

POSTER 12 – Protective role of Nrf2 pathway in mild thermotolerance and heat shock- induced apoptosis

Mélanie Grondin[1], Diana Averill-Bates[1]
[1]UQAM – Université du Québec à Montréal

POSTER 13 – IRP1 deficiency triggers metabolic reprograming, increases insulin sensitivity and protects mice against high-fat diet-induced hyperglycemia

Wen Gu[1], Nicole Wilkinson[1], Darren Blackburn[1], Korin Sahinyan[1], Vincent Richard[1], Gary Sweeney[2], Kostas Pantopoulos[1]
[1]Lady Davis Institute for Medical Research, Jewish General Hospital, and Department of Medicine, McGill University, Montreal, QC, Canada, [2]Department of Biology, York University, Toronto, ON, Canada

POSTER 14 – Role of MAFF and NRF2 transcription factors in myometrial cells: linking oxidative stress and inflammation to preterm labor

Palak Gujral[1,2], Eduardo Alonso Orozco[1,2], Xingyue Yan[1], James Saliba[1, 2], Volker Blank[1,2]
[1]Lady Davis Institute for Medical Research, [2]Department of Medicine, McGill University

POSTER 15 – Regulation of oxidative stress and inflammatory response in the intestine after infection

Shunshun Jin[1], Kathy Au-Yueng[1], Haoxiang Xu[2], Chengbo Yang[2], Karmin O[1]
[1]Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2; CCARM, St. Boniface Hospital Research Centre, Winnipeg, Manitoba, Canada, R2H 2A6., [2]Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2

POSTER 16 – Monitoring Iron-Induced Ferroptosis Using CE-ICP-DRC-MS: Principles, Applications, and Potential ex-vivo and Biofluids

Aruna Kalyanasundaram[1], Vivek Venkataramani[1], Bernhard Michalke[2]
[1]Department of Internal Medicine II, Division of Hematology and Medical Oncology, University Hospital Wuerzburg and Early Clinical Development Unit of Comprehensive Cancer Center Mainfranken, Wuerzburg, Germany, [2]Helmholtz Center Munich - German Research Center for Environmental Health GmbH, Research Unit Analytical BioGeoChemistry, Germany

POSTER 17 – Characterization of a Dehydroascorbic Acid and Homocysteine Thiolactone Reaction Product: Structural elucidation and Effect on Peptide and Protein N-Homocysteinylation

Ghizlane Loubane[1], Benazir Firdaus[1], Gabriel Robert[2], Philippe Venne[3], Christian Comeau[1], Pierre-Luc Beaudreault[1], Jeampy Komba[1], Richard J. Wagner[2], Stephen Naylor[4], Klaus Klarskov[1]
[1]Department Pharmacology and Physiology, Université Sherbrooke, [2]Département de Médecine Nucléaire et Radiobiologie, Université de Sherbrooke, [3]Chemistry department, Université Sherbrooke, [4]ReNeuroGen LLC, Milwaukee, WI, USA.

POSTER 18 – Improving oxylipin sampling and stability with solid-phase microextraction: first step towards better at-home sampling devices

Oluwatosin Kuteyi[1], Dajana Vuckovic[1]
[1]Concordia University

POSTER 19 – DUOX2 regulates secreted factors in virus-infected respiratory epithelial cells that contribute to neutrophil attraction and activation

Felix Lamontagne[1], Dacquin Kasumba[1], Elise Caron[1], Sandrine Huot[2], Audray Fortin[1], Marc Pouliot[2], Nathalie Grandvaux[1]
[1]Centre de recherche du CHUM, [2]Centre de recherche du CHU de Québec

POSTER 20 – Sex-Specific Mitochondrial Response may be Protective in the Hyperacute Phase of Neonatal Sepsis

Si Ning Liu[1], Forough Jahandideh[1], Jad-Julian Rachid[1], Claudia Holody[1], Hélène Lemieux[1], Kimberly Macala[1], Stephane Bourque[1]
[1]University of Alberta

POSTER 21 – Investigating the mechanism of myeloperoxidase-mediated toxicity of the industrial contaminant, 6-PPD, in vitro

Steven Lockhart[1], Arno Siraki[1], Dinesh Babu[1], Lusine Tonoyan[1], Newton Tran[1], Bela Reiz[1]
[1]Universiy of Alberta

POSTER 22 – Cytosolic citrate is needed for nitric oxide production during human sperm capacitation

Diego Loggia[1, 2], Cristian O'Flaherty[2, 3]
[1]Department of Pharmacology and Therapeutics, McGill University, [2]Research Institute-MUHC, [3]Departments of Pharmacology and Therapeutics, Surgery and Anatomy and Cell Biology, McGill University

POSTER 23 – Blood Ascorbic acid levels correlate with specific immune system proteins in subjects with hyperinsulinemia

Atena Mahdavi[1], Mickael Leclercq[1], Arnaud Droit[1, 2], Iwona Rudkowska[1, 3], Michel Lebel[1, 4]
[1]Endocrinology and Nephrology Unit, CHU de Québec-Laval University Research Center, Québec (QC), Canada, [2]Proteomics Platform, Centre de recherche du CHU de Québec, Faculty of Medicine, Université Laval, Québec (QC) Canada, [3]Department of Kinesiology, Faculty of Medicine, Université Laval, Québec (QC), Canada, [4]Department of Molecular Biology, Medical Biochemistry, and Pathology, Université Laval, Québec (QC), Canada

POSTER 24 –The oxidative-stress sensor TRPM2 in TNBC

Maria Maliougina[1], Yoast Ryan[2], Shekoufeh Almasi[3], Gabriela Gomes[1], Thomas Pulinilkunnil[1], Yassine El Hiani[1]
[1]Dalhousie Univeristy, [2]Pittsburgh University, [3]Fusion Pharmaceuticals

POSTER 25 – Harnessing oxidative stress imbalance to boost light-mediated therapies against pathogens

Sol Romina Martinez[1], Gonzalo Cosa[1]
[1]McGill University

POSTER 26 – Semi-targeted LC-HRMS/MS analysis of bile acids to decipher the effects of drug-induced liver injury in humans

Myriam Mireault[1], Constantine J. Karvellas[2], Christopher F. Rose[3], Lekha Sleno[1]
[1]University of Quebec in Montreal (UQAM), Chemistry department, Montreal, QC, Canada, [2]University of Alberta, Critical Care Medicine and Gastroenterology/Hepatology, Edmonton, AB, Canada, [3]CR-CHUM, Hepato-neuro laboratory, Montreal, QC, Canada

POSTER 27 – Early-life exposure to parenteral nutrition induced-peroxides changes DNA methylation and expression of antioxidants genes in guinea pigs

Angela Mungala Lengo[1], Jean-Claude Lavoie[1]
[1]Department of Nutrition, CHU Sainte-Justine, University of Montreal, Canada

POSTER 28 – Oxidising and antiproliferation properties of half-sandwich Os(II) complexes with indomethacin-functionalized ligand

Darina Muthna[1], Lukáš Masaryk[2], Eva Peterová[1], Petr Halaš[2], Alena Mrkvicová[1], Pavel Štarha[2]
[1]Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University in Prague, Šimkova 870, 500 03 Hradec Kralove, Czech Republic, [2]Department of Inorganic Chemistry, Faculty of Science, Palacky University in Olomouc, 17. listopadu 12, 77146 Olomouc, Czech Republic

POSTER 29 – Newly Discovered Sulfenylation of STAT1 Controls IFNg-Induced Gene Regulation

Cynthia Paz-Trejo[1, 2], Audray Fortin[1], Alex Harrison[1, 3], Natalia Zamorano[1, 2], Elise Caron[1], Zayd Grajales[1, 2], Nathalie Grandvaux[1, 2]
[1]CRCHUM, Montréal, QC, CA, [2]Université de Montréal, Montréal, QC, CA, [3]McGill University, Montréal, QC, CA

POSTER 30 – The impacts of cannabidiol (CBD) on mitochondrial function and redox homeostasis of trophoblast stem cells in vitro

Louise Limoges[1], Tina Podinic[2], Sandeep Raha[1]
[1]Department of Pediatrics, McMaster University, 1280 Main Street West, Hamilton ON Canada L8S 4K1, [2]Department of Pediatrics and Graduate Program in Medical Sciences, McMaster University, 1280 Main Street West, Hamilton ON Canada L8S 4K1

POSTER 31 – Targeting redox homeostasis to induce DNA damage and an inflammatory phenotype in pancreatic cancer

Emily Poulton[1, 2], Lucie Malbeteau[2], Marianne Koritzinsky[1, 2, 3, 4]
[1]Department of Medical Biophysics, University of Toronto, [2]Princess Margaret Cancer Centre, [3]Department of Radiation Oncology, University of Toronto, [4]Institute of Medical Science, University of Toronto

POSTER 32 – Impact of PERK overexpression in response to severe heat stress

Khadija Rezki[1], Diana Averill-Bates[1]
[1]UQAM - Université du Québec à Montréal

POSTER 33 – Sensitivity to Spontaneous Liver Fibrosis is Comparable in C57BL/6 and AKR Mouse Models of Hereditary Hemochromatosis

Sabrina Sgro[1], Carine Fillebeen[1], Kostas Pantopoulos[1]
[1]Lady Davis Institute for Medical Research, Jewish General Hospital, and Department of Medicine, McGill University, Montreal, QC, Canada

POSTER 34 – Targeting the Cell Surface GRP78 (csGRP78) and Anti-GRP78 autoantibodies complex in association with inflammation activity in atherosclerosis

Hitesh Sharma[1], Richard Austin[1]
[1]McMaster

POSTER 35 – CONFIDENCE: An App for Cross-Platform Differential Gene Expression Analysis and its Use in Studies of Metabolism and Oxidative Stress

Abhishek Shastry[1], Charles Hindmarch[1], Kimberly Dunham-Snary[1]
[1]Queen's University

POSTER 36 – Elevated serum fibroblast growth factor 23 predicts mortality in HIV/HCV coinfected patients

Mohamed Shengir[1], Carine Fillebeen[2], John Wagner[2], Agnihotram Ramanakumar[3], Mohammed Kaouache[3], Kostas Pantopoulos[2], Marina Klein[4], Giada Sebastiani[1, 4']
[1]Division of Experimental Medicine, Department of Medicine, McGill University, Montreal, Quebec, Canada, [2]Lady Davis Institute for Medical Research, McGill University, Montreal, Quebec, Canada, [3]Research Institute of McGill University Health Centre, Montreal, Quebec, Canada,
[4]Chronic Viral Illness Services, McGill University Health Center, Montreal, Quebec, Canada

POSTER 37 – Elucidating the role of transcriptional regulator MED15 in cancer cell oxidative stress response

Chiaki Shuzenji[1], Tiffany Chang[1], Xuanjin Cheng [1], David Liang[1], Stefan Taubert[1]
[1]University of British Colombia

POSTER 38 – Constant alcohol consumption exacerbates neurological decline and causes neuronal cell loss in rats with chronic liver disease

Farzaneh Tamnanloo[1, 2], Xiaoru Chen[2], Mélanie Tremblay[2], Christopher F. Rose[1, 2]
[1]Medicine Department, Université de Montréal, Montréal, Canada, [2]Hepato-neuro lab, CRCHUM, Montréal, Canada

POSTER 39 – Time-dependent changes to skeletal muscle macrophage redox homeostasis in Myositis
Arshdeep Thuhan[1], Madison Garibotti[1], Ramsha Mansuri[1], Parsa Vahabishekarloo[1], Ali Abdul Sater[1], Christopher Perry[1]
[1]York University

POSTER 40 – Impact of Covid-19 Antiviral Drugs on Neutrophil Oxidative Activity
Lusine Tonoyan[1], Dinesh Babu[2], Steven Lockhart[2], Béla Reiz[3], Arno Siraki[2]
[1]Applied Pharmaceutical Innovation, Edmonton, AB, [2]College of Health Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, [3]College of Natural and Applied Sciences, Faculty of Science, Department of Chemistry, University of Alberta

POSTER 41 – Cre-dependent Sod1 knockout in mouse embryonic fibroblasts
Long Truong-Ong[1], Ying Wang[1], Siegfried Hekimi[1]
[1]McGill University

POSTER 42 – Transferrin receptors 1 and 2 are dispensable for iron supply to hepatocytes
Sofiya Tsyplenkova[1], Edouard Charlebois[1], Nathan Subramaniam[2], Carine Fillebeen[1], Kostas Pantopoulos[1]
[1]Lady Davis Institute for Medical Research, Jewish General Hospital, and Department of Medicine, McGill University, Montreal, QC, Canada, [2]Queensland University of Technology, Kelvin Grove, QLD, Australia

POSTER 43 – Evaluating the role of lipid-derived electrophiles in ferroptotic cell death using fluorescence microscopy
Antonius Van Kessel[1], Gonzalo Cosa[1]
[1]Department of Chemistry, McGill University

POSTER 44 – The inefficacy of oral Coenzyme Q10 in treating primary CoQ deficiency and the solution to the problem
Ying Wang[1], Siegfried Hekimi[1]
[1]Biology Department, McGill University

POSTER 45 – Attenuation of Oxidative Stress and Improving Kidney Function by Folate in Acute Kidney Injury
Charith Wijerathne[1, 2], Kathy Au-Yeung [1, 2], Siow Yaw[3, 4], Karmin O[1, 2, 3]
[1]Department of Animal Science, University of Manitoba, [2]St. Boniface Hospital Research Centre, Winnipeg, [3]Department of Physiology & Pathophysiology, University of Manitoba, [4]Agriculture and Agri-Food Canada

POSTER 46 – Nuclear-Mitochondrial DNA Mismatch Induces Tissue-Specific Gene Expression Profiles: Transcriptomic Analysis of Subcutaneous and Visceral White Adipose Tissues in Mice
Mia Wilkinson[1], Abhishek Shastry[1], Charles Hindmarch[1], Kimberly Dunham-Snary[1]
[1]Queen's University

POSTER 47 – Role of the transcription factor NFE2L3 in a mouse model of inflammation: link to colorectal cancer sidedness and oxidative stress
Linda Yaker[1], James Saliba[1], Liam Scott[1], Anantpreet Kaur Sood [1], Volker Blank[1]
[1]Lady Davis Institute for Medical Research and Department of Medicine, McGill University, 3755 Cote Sainte-Catherine Road, Montreal, Quebec, Canada, H3T 1E2.

POSTER 48 – ER retention of proPCSK9 protects against cardiometabolic disease risk in in vitro and in vivo models
[1]Paul Lebeau1, Jae Hyun Byun1, Richard Austin1
1Department of Medicine, Division of Nephrology, McMaster University, St. Joseph's Healthcare Hamilton, Ontario L8N 4A6, Canada

POSTER 49 – The role of oxidative stress in dietary carbohydrate and manganese-induced hepatic lipid metabolism in fish
Tao Zhao[1,2], Kostas Pantopoulos[2], Zhi Luo[1,*]
[1]College of fisheries, Huazhong Agricultural University, Wuhan, Hubei, China
[2]Lady Davis Institute for Medical Research, Jewish General Hospital, and Department of Medicine, McGill University, Montreal, QC, Canada

POSTER ABSTRACTS
by Primary Authors – 1 to 49

POSTER 1 – Role Of Bioactive Compounds On Sodium Iodate Induced Retinal Pigment Cell Damage

Nicholas Bel[1], Sanjoy Gupta[2], Neelam Khaper[1, 2]

[1]Lakehead University, Thunder Bay, ON, [2]Northern Ontario School of Medicine University, Thunder Bay, ON

Age-Related Macular Degeneration (AMD) is one of the leading causes of blindness in the developed world. AMD is known to be a multifactorial disease in which oxidative stress plays a role in its progression and leads to a rapid deterioration of vision. Reactive oxygen species (ROS) that accumulate can cause damage within the layers of the eyes, specifically in the retinal pigment epithelial (RPE) cells. In-vitro, sodium iodate (SI) has been used to simulate the changes that occur to the RPE cells during the development and progression of AMD. Antioxidants are known to combat the damaging effects of ROS. Lutein and Zeaxanthin are naturally occurring xanthophyll compounds from vegetables which have been reported to possess antioxidant properties. In our study, SI alone induced a significant reduction in viability of ARPE-19 cells (a human RPE cell line). When pre-treated with lutein (25uM) in the presence of 17.5mM SI, cellular viability was improved by 22% compared to SI alone. Pre-treatment of zeaxanthin (10uM) also resulted in an improvement in cell viability when exposed to 15mM and 17.5mM of SI. The H2DCFDA assay was used to quantify intracellular oxidative stress present within the RPE cells. When exposed to SI, there was an increase in oxidative stress which was attenuated by pre-treatments with both lutein and zeaxanthin. In order to gain a mechanistic insight by which these compounds garner protection from the damaging effects of SI, CRISPR knockout (KO) ARPE-19 cells were created. Sirtuin-1 (SIRT-1) is a well-known modulator of ROS and our preliminary experiments with SIRT-1 KO cells show an increase in susceptibility to oxidative damage caused by SI when compared to wild type cells. Pre-treatment with zeaxanthin attenuated SI mediated damage in SIRT-1 KO cells when compared to the controls of SI alone. Future studies are directed towards characterizing the effects of these compounds on markers of oxidative stress and cell death.

POSTER 2 – Stimulation of RAS-dependent ROS signaling extends longevity by modulating a developmental program of global gene expression

Robyn Branicky[1], Ying Wang[1], Arman Khaki[1], Maximilian Kramer-Drauberg[1], Siegfried Hekimi[1]

[1]McGill University

We show that elevation of mitochondrial superoxide generation increases C. elegans lifespan by enhancing a RAS-dependent ROS signaling pathway (RDRS) that controls the expression of half of the genome as well as animal composition and physiology. RDRS stimulation mimics a program of change in gene expression that is normally observed at the end of post-embryonic development. We further show that RDRS is regulated by negative feedback from the SOD-1-dependent conversion of superoxide into cytoplasmic hydrogen peroxide, which in turn acts on a redox-sensitive cysteine (C118) of RAS. Preventing C118 oxidation by replacement with serine, or mimicking oxidation by replacement with aspartic acid, leads to opposite changes in the expression of the same large set of genes that is affected when RDRS is stimulated by mitochondrial superoxide. The identities of these genes suggest that stimulation of the pathway extends lifespan by boosting turnover and repair while moderating damage from metabolic activity.

POSTER 3 – TXNIP promotes ferroptosis through NCOA4 mediated ferritinophagy

Pandian Nagakannan[1], Shakila Sultana[1], Md Imamul Islam[1], Eftekhar Eftekharpour[1]

[1]Dept. Physiology and Pathophysiology, University of Manitoba

Introduction:

Ferroptosis is a form of oxidative cell death mechanism driven by the accumulation of lethal lipid hydroperoxides. In addition to iron mediated oxidation of phospholipids, depletion of lipid peroxide detoxifying antioxidant glutathione peroxidase-4 (GPX4) and its substrate glutathione (GSH) can also induce ferroptosis. Dysregulated ferroptosis has been linked to many diseases including cancer, tissue injury, neurodegeneration, inflammation, and infection. We have previously shown the executive role of lysosomal cathepsin B in induction of ferroptotic cell death. Upregulation of Thioredoxin-Inhibiting Protein (Txnip) has also been linked to lysosomal permeability and cathepsin B release. In this project we aimed to examine the impact of TXNIP in ferroptotic cell death.

Methods: Immortalized mouse embryonic fibroblast (MEF), hippocampal neuronal cells (HT22), human cervical cancer cells (HeLa) and their TXNIP-deficient counterparts were used for testing their response to the classic ferroptosis inducing agents.

Results: Here, we show that TXNIP deficient fibroblasts, immortalized neuronal cells and cervival cancer cells were resistant to ferroptosis inducing agents such as erastin, glutamate, RSL3 and ML210. In contrast, overexpression of TXNIP sensitized cells to ferroptotic cell death. Moreover, $TXNIP^{-/-}$ cells were resistant to mitochondrial dysfunction caused by ferroptotic inducers and the protection observed was not dependent on GSH and GPX4 status. Mechanistically, TXNIP promoted the degradation of iron binding protein, ferritin through NCOA4 (Nuclear Receptor Coactivator 4) mediated ferritinophagy. This selective autophagic degradation of ferritin increased the cytosolic labile iron pool, thereby enhancing lipid peroxidation and ferroptosis.

Conclusion: Collectively, our results suggest TXNIP as a positive regulator of ferroptosis by controlling autophagy and iron availability. Manipulation of TXNIP may be a potential target for the development of drugs for treating diseases targeting ferroptotic pathway.

POSTER 4 – Characterizing partners of the C. elegans Nuclear Hormone Receptor NHR-49 in oxidative stress response

Glafira Ermakova[1], Kelsie Doering[1], Stefan Taubert[1]

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Oxidative stress occurs when there is an accumulation of Reactive Oxygen Species (ROS) that can damage DNA, protein, lipids, and organelles such as mitochondria, causing cellular stress. The pathways that respond to oxidative stress are thus important for cellular survival, and studying them can help understand the formation of diseases such as cancer, which feature oxidative stress. Oxidative stress response pathways are highly conserved throughout evolution. For example, the human NF-E2-Related Factor 2 (Nrf-2) is homologous to the C. elegans transcription factor Skinhead-1 (SKN-1), and both proteins play a key role in oxidative stress defense. Nuclear hormone receptors (NHRs) are another group of C. elegans transcription factors (TFs) that regulate physiological and processes. Our lab previously showed that NHR-49 is required for oxidative stress responses in C. elegans, and that it acts in parallel to the canonical SKN-1 pathway. However, in contrast to the SKN-1 pathway, the C. elegans NHR-49 oxidative stress signaling network remains poorly characterized. To identify genes that may act within the NHR-49 oxidative stress signaling pathway, we performed a reverse genetic RNAi screen using the NHR-49-dependent, stress-inducible fmo-2p::GFP reporter. This screen identified several TFs, which may act within the NHR-49 controlled oxidative stress response pathway. To test whether these genes act with nhr-49, I am currently studying these candidate TFs to determine whether their loss or gain of function phenocopies nhr-49 knockout, i.e., loss causing oxidative stress sensitivity, and gain causing stress resistance. I am also performing genetic interaction studies of these genes with nhr-49 to test whether they act in the same genetic pathway. Thus, my work provides new insight into the makeup and complexity of the NHR-49 oxidative stress network and helps characterize the molecular response to stress, thereby providing us with new potential cancer drug targets.

POSTER 5 – Beta-caryophyllene effects in pulmonary oxidative stress in pulmonary hypertensive rats

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Pulmonary arterial hypertension (PAH) is related to increased oxidative stress. Thus, the use of antioxidants, such as beta-caryophyllene, could represent an adjuvant treatment for this disease. Furthermore, the use of a nanoemulsion of this compound could be more effective by increasing its bioavailability. This study aimed to determine the effects of free and nanoemulsion beta-caryophyllene in some aspects of PAH, such as pulmonary vascular resistance (PVR), oxidative stress and right ventricular hypertrophy. PAH in rats was induced by MCT (60 mg/kg intraperitoneally) and, 7 days later, treatment with beta-caryophyllene or nanoemulsion (by gavage, 176 mg/kg/day) or vehicle was given for 14 days. Male Wistar rats (170g, n=6/group) were divided into four groups: control (CO), monocrotaline (MCT), monocrotaline + beta-caryophyllene (MCT-Bcar) and monocrotaline + nanoemulsion with beta-caryophyllene (MCT-Nano). Mean pulmonary arterial pressure (mPAP), right ventricle end diastolic pressure (RVEDP), right ventricle systolic pressure (RVSP), and PVR, evaluated by echocardiographyic measurements, were performed and, after, rats were killed by decapitation. Right ventricle (RV) was removed for morphometry, and lungs to evaluate oxidative stress parameters (TBARS), antioxidants (sulfhydryls and antioxidant enzymes: SOD, CAT and GPx) and the balance between nitric oxide (NO) and reactive oxygen species (ROS). Treatment with free or nanoemulsion of beta-caryophyllene increased the activities of SOD and CAT, as well as reduced lipid peroxidation, and improved the NO/ROS balance in the lungs of MCT animals. Furthermore, there was a significant improvement in both, flow and pressure in the pulmonary artery in the groups treated with both beta-caryophyllene presentations. However, only the nanoemulsion formulation of this compound was able to increase the concentration of total sulfhydryls and GPx activity, to reduce RV hypertrophy, RVEDP and RVSP, and to improve RV systolic function (TAPSE) in rats with PAH. Beta-caryophyllene has beneficial effects on PAH, being able to reduce oxidative stress. However, the nanoemulsion of this compound showed more promising results, being able to raise the levels of all antioxidants tested, and especially, to improve RV systolic function, which is a predictive factor in patients with PAH.

POSTER 6 – Hepcidin inhibits iron efflux from duodenal ferroportin and thereby indirectly promotes iron-dependent DMT1 degradation

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Background and aims. Hepcidin is a liver-derived peptide hormone that controls systemic iron homeostasis by inhibiting iron entry into plasma. It binds to the iron exporter ferroportin triggering its degradation. Herein, we studied functional properties of synthetic hepcidin in mice.
Methods. Wild type and hemojuvelin (Hjv)$^{-/-}$ mice, a model of hemochromatosis due to hepcidin deficiency, were injected with synthetic hepcidin. Pharmacokinetic data were obtained in plasma. Expression of ferroportin and other iron metabolism proteins was monitored in target tissues.
Results. A dose of 2 mg/kg synthetic hepcidin reached maximal plasma concentration of 1.3 ng/ml, with ~70 min half-life and decreased plasma iron in wild type mice within 4 h. Hjv$^{-/-}$ mice exhibited significant drop in plasma iron after two consecutive hepcidin injections within 8 h. Synthetic hepcidin diminished ferroportin levels in the spleen and liver of wild type and Hjv$^{-/-}$ mice. Hepcidin also partially decreased expression of ferroportin and the apical metal transporter DMT1 in wild type duodenal enterocytes. Surprisingly, synthetic hepcidin profoundly suppressed duodenal divalent metal transporter 1 (DMT1) in Hjv$^{-/-}$ mice without affecting highly induced ferroportin. Nevertheless, the treatment increased duodenal iron content. Experiments in intestinal mouse organoids and polarized human Caco-2 cells showed that hepcidin indirectly promotes DMT1 degradation via iron accumulation.
Conclusions: Pharmacological doses of hepcidin modulate iron homeostasis in wild type and Hjv$^{-/-}$ mice by degrading splenic and hepatic ferroportin. Hepcidin also degrades basal duodenal ferroportin but may be limiting when ferroportin is overexpressed. Nevertheless, hepcidin occludes ferroportin's iron export channel, which in turn promotes iron retention and iron-dependent DMT1 degradation.

POSTER 7 – Real-time monitoring of antibiotic-triggered lipid peroxidation in bacterial membranes

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The ability of bacteria to trigger the formation of membrane vesicles (MVs) has captivated the scientific community in the past decade. MVs are spherical buds of the bacterial outer membrane which contain periplasmic components from the donor cell. MVs are postulated to facilitate bacteria communication, virulence, and the emergence of antibiotic resistance upon MVs formation under antibiotic-induced oxidative stress.

This presentation will explore the role oxidative stress plays on MVs formation in Gram-negative Escherichia coli, through lipid peroxidation elicited by antibiotic-induced oxidative stress. Working with a fluorogenic α-tocopherol analogue, a lipid peroxyl radical sensor probe, we will first show bacterial membrane damage in the presence of an antibiotic via ROS-induced lipid peroxidation. We will also show bacterial membrane blebbing associated with membrane oxidation.

This work opens new venues to the mechanistic understanding of the role of lipid peroxidation in the formation of bacterial MVs.

POSTER 8 – Exploring the effects of an experimental autoimmune myositis rodent model on mitochondrial H2O2 emission in skeletal muscle

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Introduction: Myositis is a rare autoimmune disorder that causes skeletal muscle inflammation and weakness. A recent report identified increased muscle mitochondrial H_2O_2 emission in patients and a mouse model of myositis using a mixed glutamate (NADH) and succinate ($FADH_2$) substrate protocol. However, the degree to which skeletal muscle ROS is generated from forward or reverse electron transfer, respectively, during myositis remains unknown.

Methods: 12-16-week-old Female BALB/c mice were used to induce a mouse model of experimental autoimmune myositis (EAM). All animals received one injection every seven days, for a total of four injections, and sacrificed seven days after the final injection (total period: 28 days). Treatment animals received in-lab purified rabbit myosin stored in glycerol with Freund's Complete Adjuvant (CFA) for the first injection, and Freund's Incomplete Adjuvant (IFA) for the remaining three injections to enhance the immune response to myosin. Two separate control groups received either glycerol at each time point or CFA as the first injection with IFA injections at the remaining time points. Skeletal muscle tissue was collected and used to prepare permeabilized muscle fibers for assessments of complex I forward (NADH; pyruvate/malate) and reverse electron transfer ($FADH_2$; succinate) supported H_2O_2 emission, under state II conditions (no ADP). ADP was then titrated (state III) to permit forward electron flow to complex IV in all substrate conditions and to determine the ability of ADP to attenuate H_2O_2.

Results: In diaphragm fibers, there were no differences in state II H_2O_2 emission in response to any substrate. However, under state III conditions, reverse electron transfer supported mitochondrial H_2O_2 emission was increased ~13% in the EAM group compared to the glycerol CFA/IFA control group ($p=0.0261$), demonstrating an impaired ability of ADP to attenuate H_2O_2 emission. ADP had no effect on forward electron transfer supported H_2O_2 emission.

Conclusion/next steps: This EAM mouse model demonstrated an impaired ability of ADP to attenuate H_2O_2emission in a substrate-specific manner in diaphragm muscle fibres. This ongoing project will relate these responses to substrate-specific mitochondrial respiration and contents, autoimmunity markers and assessments of muscle force production and fibre size throughout the progression of myositis.

POSTER 9 – Untargeted and targeted metabolomics by LC-MS/MS for studying metabolic perturbations in biological samples

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Metabolomics aims to study the variation of metabolites in biological samples. The understanding of metabolic variation is an important step towards a better understanding of diseases and environmental perturbations. Metabolic changes caused by external or internal stimuli, such as oxidative stress, often occur before clinical signs appear. To study the metabolic variations of different biological samples, a "ready to use" commercial kit was tested to access over 600 metabolites. The samples are prepared with a derivatization step using phenyl isothiocyanate, followed by extraction. Targeted and quantitative LC-MRM analyses on a triple quadrupole platform are then employed to access both polar metabolites and lipids. To increase metabolic coverage, an untargeted high-resolution tandem mass spectrometry method was also developed on a quadrupole-time of flight platform. To validate metabolite identification from this untargeted data, an in-house spectral library has been built from the analysis of over 200 metabolite standards. This poster will contrast the advantages of both targeted and untargeted approach for studying the metabolic response to perturbations involving oxidative stress induced by disease or environmental factors.

POSTER 10 – Examining the effects of 8-oxoguanine on gene expression and mitochondrial biology

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8-oxoguanine (8-oxoG) is a common oxidative modification found in DNA. Conventionally recognized as a form of damage, mounting evidence suggests that 8-oxoG functions as an epigenetic modification. Of particular interest, 8-oxoG has been shown to modulate gene expression through its repair enzymes, Ogg1 and Ape1, which facilitate the localization of transcription factors and epigenetic regulators to repair sites. We have recently found that 8-oxoG is enriched in gene promoters, and that cells cultured at different oxygen levels harbor unique subsets of genes with oxidized promoters. This suggests that targeted promoter oxidation may be a regulatory mechanism by which cells alter transcription to adapt to oxygen availability. Currently, we are investigating how oxygen-dependent promoter oxidation affects gene expression, and probing the involvement of the base-excision repair process through siRNA knockdowns of Ogg1 and Ape1. While 8-oxogaunine has been well studied in DNA, it is more abundant in RNA, though less is known about its potential regulatory function in this context. We plan to survey 8-oxoG in the human transcriptome, with interest in how its placement within transcripts regulates mRNA fate. In addition, our lab has focused on how the molecular biology of cells is broadly affected by culture in various oxygen conditions, and in particular, physioxia. Physioxia comprises the 2-11% range of oxygen levels characteristic of most human tissues, and may provide a more physiologically relevant setting than normoxia (21% oxygen), at which cell culture is typically performed. Interestingly, we showed that mitochondria exhibit higher metabolic activity and a greater degree of fusion in physioxia compared to normoxia, suggesting that the mitochondrial network is ‘healthier’ in the physioxic range. Future work will dive deeper into mitochondrial biology to investigate how physioxia affects mitochondria number and the process of mitophagy. This research will contribute to the growing body of evidence that the molecular and cellular biology of cells cultured in physioxia is different, and that oxygen should be considered as a cell culture parameter if making physiological inferences.

POSTER 11 – An investigation into the effect of sex on the hydrogen peroxide (H2O2) generating capacities of individual sites of production in mouse liver mitochondria

Cathryn Grayson[1], Olivia Koufos[1], Ryan Mailloux[1]

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Mitochondria produce hydrogen peroxide (H_2O_2) at 16 sites, 12 of which are associated with fuel combustion pathways and the electron transport chain (ETC). Pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (αKGDH), and complex III, are documented as the main liver mitochondrial H_2O_2 sources that metabolize pyruvate and citric acid (TCA) cycle metabolites. Complexes I and III are also primary H_2O_2 sources when nutrients that donate electrons directly to the coenzyme Q_{10} (CoQ) pool fuel respiration. This study is the first to show sex differences in H_2O_2 production at individual sites based on male and female C57BL/6N mouse liver mitochondria. During pyruvate and malate oxidation, primary sites of H_2O_2 production were found to be αKGDH and PDH for both sexes. However, succinate oxidation differed according to sex (complex I was the main H_2O_2 source in male mitochondria, while female mitochondria used complexes I and III equally). During fatty acid oxidation, only male mitochondria produced as much as ~50% of its H_2O_2 at complex III. It's also revealed for the first time that while oxidizing fatty acids and malate, αKGDH was the primary H_2O_2 generator in both males and females. Statistically, we found the main H_2O_2 sources in fatty acid oxidation were αKGDH and complex III in males, while females used αKGDH and complex I. Overall, female liver mitochondria were found to produce less H_2O_2 than males when oxidizing any substrate. And, of any substrate/sex combination, male mitochondria oxidizing fatty acids produced the most H_2O_2. In summary, we demonstrated for the first time that αKGDH is the sex independent primary H_2O_2 source during fatty acid metabolism. We also showed that other previously established primary H_2O_2 sources are sex dependent. This novel work adds a new dimension to understanding mitochondrial H_2O_2 budgeting—largely, that αKGDH is one of, if not the main H_2O_2 generator overall.

POSTER 12 – Protective role of Nrf2 pathway in mild thermotolerance and heat shock-induced apoptosis

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Exposure to low doses of stress induces adaptive survival responses that protect cells against subsequent exposure to cytotoxic stress (e.g. oxidative stress, heat shock, hypoxia). The ability of cells to resist subsequent cytotoxic stress following exposure to low-dose heat stress at 40°C is known as mild thermotolerance. Mild thermotolerance involves increased expression of heat shock proteins and antioxidants, but the initiating factors in this response are not understood. Nrf2 is a transcription factor that plays a key role in regulating the expression of genes involved in the antioxidant response and cellular detoxification. This study aims to understand the role of the Nrf2 antioxidant pathway in the acquisition of mild thermotolerance at 40°C, and secondly, whether the Nrf2 pathway could be involved in the protective effect of thermotolerance against heat-shock (42°C)-induced apoptosis. This study investigated the role of the Nrf2 pathway in regulating autophagy and apoptosis in response to moderate heat stress. Our results showed that HeLa cells with knocked down (KD) expression of Nrf2 were more susceptible to autophagy and apoptosis when exposed to heat stress compared to WT cells. This suggests that Nrf2 is involved in regulating these cellular processes in response to heat stress. Furthermore, our findings showed that the increased susceptibility to cell death at 42°C in Nrf2 KD cells could be attributed to increased oxidative stress, which in turn activates the autophagy and apoptosis signaling pathways. There was an increase in lysosomal activity at 40°C in Nrf2 KD cells, which may contribute to the increased sensitivity to cell death. In addition, levels of peroxides were higher in Nrf2 KD cells. Finally, the addition of antioxidants such as PEG-Catalase and PEG-SOD appeared to decrease the impact of heat stress on both KD and WT cells, further supporting the role of oxidative stress in this stress response. These findings suggest that disruption of the Nrf2 pathway may affect antioxidant responses and cellular detoxification, which may have significant consequences for human health.

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POSTER 13 – IRP1 deficiency triggers metabolic reprograming, increases insulin sensitivity and protects mice against high-fat diet-induced hyperglycemia

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Iron regulatory protein 1 (IRP1) is a post-transcriptional regulator of cellular iron metabolism. While IRP1 operates as cytosolic aconitase in iron-replete cells, iron deficiency promotes its conversion to an RNA-binding protein that controls expression of mRNAs containing "iron responsive elements" (IREs). $Irp1^{-/-}$ mice develop polycythemia due to unrestricted translation of HIF2α mRNA, an IRP1 target, which in turn stimulates erythropoiesis via transcriptional induction of erythropoietin. We observed that $Irp1^{-/-}$ mice exhibit fasting hypoglycemia. Moreover, the male animals are protected against hyperglycemia that develops in wild type littermates in response to a high fat diet. This is due to insulin-mediated decreased gluconeogenesis in the liver and increased glucose uptake in skeletal muscles. Mechanistically, decreased gluconeogenesis is caused by induction of insulin receptor substrate 2 (IRS2), a HIF2α target in the liver, which increases insulin sensitivity. Nevertheless, without insulin stimulation, expression of gluconeogenic G6pc mRNA was induced in $Irp1^{-/-}$ mice. Furthermore, expression of Srebp1 mRNAs, which encodes a gene involved in de novo lipogenesis, was suppressed. Proteomics analysis revealed mitochondrial dysfunction and metabolic reprogramming in the liver and skeletal muscles of $Irp1^{-/-}$ mice. Seahorse assays using primary hepatocytes and differentiated myotubes validated the impaired respiratory capacity and switch to glycolytic metabolism. Our data suggest that mitochondrial dysfunction decreases energy production in skeletal muscles, which triggers increased insulin sensitivity and glucose uptake. However, the energy yield is low, and starvation signals stimulate expression of gluconeogenic genes and suppress lipogenesis in the liver overriding high insulin sensitivity, in a futile attempt to meet energetic needs. Thus, IRP1 emerges as an important metabolic regulator.

POSTER 14 – Role of MAFF and NRF2 transcription factors in myometrial cells: linking oxidative stress and inflammation to preterm labor

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Background: Intrauterine infections account for approximately 30-40% of cases of spontaneous preterm labor, prompted by untimely activation of proinflammatory cytokines. We have previously identified the MAFF basic leucine zipper transcription factor as an interleukin-1 beta (IL1B) and tumor necrosis factor alpha (TNF) cytokine induced protein in uterine smooth muscle (myometrial) cells. We also found that MAFF, in turn, positively controls CXCL1 chemokine and CSF3 cytokine levels. Literature reports have shown that MAFF functions as heterodimeric partner of the NRF2 transcription factor, a key regulator of oxidative stress. We hypothesize that MAFF function and cytokine signaling in myometrial cells are linked to oxidative stress.

Methodology: PHM1-31 myometrial cells were treated with IL1B (10 ng/mL) for 4hrs. We have knocked down MAFF and NRF2 in PHM1-31 cells using siRNA and/or lentiviral-based transduction of shRNA. Knockdown of MAFF and NRF2 and expression of candidate target genes was verified using qPCR and immunoblot assay.

Results: We observed the induction of superoxide dismutase 2 (SOD2) transcripts in IL1 B-treated myometrial cells. This is of interest, as SOD2 clears reactive oxygen species and functions as a protector against oxidative stress and proinflammatory cytokines. We will further analyze the effect of MAFF and NRF2 knockdown on SOD2, HMOX1, FTH1, PRDX6, RXRα, and AKR1B1 gene expression both in the absence and presence of IL1B treatment.

Conclusion: IL1B functions as an inducer of ROS and SOD2 levels in uterine smooth muscle PHM1-31 cells. SOD2 thus contributes to the protection of myometrial cells against oxidative stress and cell death. The MAFF and NRF2 transcription factors may play an important role in the control of the oxidation and reduction equilibria in myometrial cells by regulating the expression of antioxidant genes.

POSTER 15 – Regulation of oxidative stress and inflammatory response in the intestine after infection

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Enterotoxigenic Escherichia coli (ETEC) is a bacterial pathogen commonly associated with gastrointestinal disease and a major contributor to global morbidity and mortality. Additionally, travelers' diarrhea is a common complication of ETEC infection. Oxidative stress can impair nutrient absorption and the integrity of the intestine. Avilamycin has been found to be effective in controlling stress-induced post-weaning diarrhea in piglets primarily caused by ETEC infection. The present study aimed to investigate the regulation of oxidative stress and inflammatory response in a piglet model challenged with ETEC and to determine if avilamycin supplementation could improve intestinal function in piglets. The weaned piglets susceptible to ETEC F4 were randomly divided into three groups: control, ETEC challenge (5 mL of 5 × 10^6 CFU/mL ETEC F4), and ETEC challenge plus avilamycin for five days. ETEC infection significantly increased serum and intestinal (jejunum) malondialdehyde (MDA) levels and diamine oxidase (DAO) activity while reducing glutathione (GSH) levels and the ratio of GSH to GSSG in the intestine. ETEC infection also significantly decreased nuclear factor erythroid 2-related factor 2 (Nrf2), tight-junction protein ZO1, superoxide dismutase-1 (SOD), and heme oxygenase-1 (HO-1) expression. Furthermore, ETEC infection significantly increased the expression of pro-inflammatory cytokines (IL-6 and TNF-α) in the intestine. Avilamycin supplementation reduced oxidative stress and inflammation, as evidenced by decreased MDA levels, increased GSH levels, increased expression of SOD, HO-1, and nuclear Nrf2, and decreased expression of IL-6 and TNF-α. Avilamycin supplementation also improved intestinal integrity, as demonstrated by decreased DAO activity and increased ZO1 expression. These results suggest that attenuation of oxidative stress may improve intestinal function after infection.

POSTER 16 – Monitoring Iron-Induced Ferroptosis Using CE-ICP-DRC-MS: Principles, Applications, and Potential ex-vivo and Biofluids

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Ferroptosis is a distinct form of regulated cell death that occurs when membrane lipid peroxidation is unrestrained. This process is tightly regulated by various factors related to cell metabolism, including the availability and compartmentalization of redox-active iron (Fe^{2+}). Reliable quantitative methods for Fe^{2+}/Fe^{3+} redox-speciation in body fluids are crucial to assess the risk of injured tissue undergoing ferroptosis.

We introduce a novel "one pot-two shot" method that involves two analytical steps from the same sample using different analytical parameters. By changing buffer chemicals, different separation conditions can be achieved for either iron speciation or sulfur and selenium speciation. During the second shot, GSH and GSSG are quantified via respective sulfur content, while GPX4, a seleno-protein, is quantified via selenium.

The Fe^{2+}/Fe^{3+} ratio in whole cell extracts accurately reflects changes in the cellular labile iron pool (LIP), while the shift in GSH/GSSG ratio reflects the exhaustion of metabolic ROS protection. We successfully applied this method in cell and tissue lysates, as well as in various biofluids, including cerebrospinal fluid and bone marrow plasma. Our method demonstrates high accuracy (97-105% recovery) and a low detection limit of ~3.5 µg/L for iron species and 10 mg/L for sulfur and selenium species, a valuable tool for the quantitative assessment of ferroptosis in various biological systems.

POSTER 17 – Characterization of a Dehydroascorbic Acid and Homocysteine Thiolactone Reaction Product: Structural elucidation and Effect on Peptide and Protein N-Homocysteinylation

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An excessive blood level of homocysteine (HcySH) is associated with numerous cardiovascular and neurodegenerative disease conditions. It has been suggested that direct S-homocysteinylation, of proteins by HcySH, or N-homosteinylation by homocysteine thiolactone (Hctl) could play a causative role in these maladies. In contrast, ascorbic acid (AA) plays a significant role in oxidative stress prevention. AA is oxidized to dehydroascorbic acid (DHA) and if not rapidly reduced back to AA may degrade to reactive carbonyl products. In the present work, DHA is shown to react with Hctl to produce 3a,6-dihydroxy-2-oxotetrahydro-2H-spiro[furo[3,2-b]furan-3,2'-[1,3]thiazinane]-4'-carboxylic acid. This reaction product is formed by initial creation of a carbinolamine, or imine product with C2 of DHA followed by hydrolysis of the Hctl thioester and anchimeric assistance of the resulting thiol anion to form a 1,3-thiazinane-4-carboylic acid moiety. The reaction product was determined to have an accurate mass of 291.0415 and a molecular composition of $C_{10}H_{13}NO_7S$ containing five double bond equivalents. We structurally characterized the reaction product using a combination of accurate mass tandem mass spectrometry and 2D-nuclear magnetic resonance. We also demonstrate that the reaction product impeded peptide and/or protein N-homocysteinylation by Hctl using model peptides and α-lactalbumin. Furthermore, the reaction product is formed in Jurkat cells when exposed to Hctl and DHA. Paradoxically, our results suggest that oxidative stress and hence an elevated level of DHA could protect against the potential damaging protein modifications by Hctl.

POSTER 18 – Improving oxylipin sampling and stability with solid-phase microextraction: first step towards better at-home sampling devices

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Innovative at-home sampling devices, such as dried plasma collection cards and microneedle-based devices, have enhanced biospecimen collection techniques by reducing the invasiveness of sample collection and allowing user to self-collect a sample of appropriate quality for subsequent analysis. COVID-19 pandemic has further accelerated the adoption of at-home sampling and biospecimen collection for numerous applications. However, existing at-home sampling approaches may not perform well for unstable biomarkers including oxidative stress biomarkers. Oxylipins are the oxidized products of polyunsaturated fatty acids (PUFAs) and can be produced enzymatically or by reaction with reactive oxygen species (ROS). Oxylipins play many significant roles in humans including in immunity and inflammation and are potential biomarkers of disease. The measurement of oxylipins is challenging due to their poor stability during sampling, transportation, and storage. In vivo solid-phase microextraction (SPME) is a promising extraction technique for oxylipins, which integrates sampling and sample preparation into a single step while preserving oxylipins from enzymatic degradation post-sampling. The goal of this study was to investigate if SPME devices can also protect oxylipins from non-enzymatic degradation during the freeze-and-thaw process or room temperature storage for 18 days. Oxylipins were extracted using HLB SPME devices from standards or human plasma. Stability was evaluated by comparing all tested conditions against freshly extracted samples (t=0) using the acceptance criteria of 80-120% and ANOVA statistical analysis. In the 3-cycle freeze-and-thaw stability study in standards, 5-hydroxyeicosapentaenoic acid, prostaglandin E2 (PGE2), and prostaglandinF2α ethanolamide increased while 15-hydroxy-11Z,13E-eicosadienoic acid, and arachidonic acid decreased. In plasma, 11β-prostaglandin F2α, 8-iso-15(R)-prostaglandin F2α, prostaglandin F2α, and several hydroxydocosahexaenoic and hydroxyeicosapentaenoic acids were unstable. At room temperature, out of 40 oxylipins tested, only 4-hydroxydocosahexaenoic acid was unstable. This result is the first to show that SPME can effectively improve the stability of this class of compounds during storage and transportation. These findings open up new possibilities to incorporate microextraction into at-home sampling devices and enable better sampling of oxylipins and other oxidative stress markers.

POSTER 19 – DUOX2 regulates secreted factors in virus-infected respiratory epithelial cells that contribute to neutrophil attraction and activation

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Purpose: Respiratory epithelial cells (ECs) produce the earliest elements of the immune response mainly through the secretion of antiviral and proinflammatory cytokines. The cytokine response not only restricts virus replication and dissemination, but also orchestrates the subsequent immune response. The epithelial NADPH dual oxidase 2 (DUOX2) is induced upon virus infection in ECs and was found to be essential for a sustained interferon response, which is key to limit virus replication. Here, we investigated the role of DUOX2 in the inflammatory arm of the cytokine response. Experimental design: A549 alveolar epithelial cells deficient in DUOX2 were generated using CRISPR-Cas9 and infected with Sendai virus (SeV). Profiling of cytokines was performed by multiplex Luminex-based assays. The impact of epithelial DUOX2-deficiency on neutrophils activation was assessed by using an ex-vivo system in which isolated primary human neutrophils were exposed to conditioned media. Neutrophils viability, chemotaxis, ROS production, formation of extracellular traps (NET), and surface expression of adhesion and degranulation markers were monitored. Results: We found that the absence of epithelial DUOX2 selectively reduced the induction of a restricted panel of 14 cytokines and chemokines secreted in response to SeV infection by 20 to 89%. The secreted factors produced by ECs upon virus infection promoted the migration, adhesion and degranulation of primary human neutrophils, in part through the DUOX2-dependent secretion of TNF and other chemokines. In contrast, DUOX2 expression did not impact neutrophil viability or NETosis, thereby highlighting a selective impact of DUOX2 in neutrophil functions. Conclusion: Overall, this study unveils previously unrecognized roles of epithelial DUOX2 in the epithelial-immune cells crosstalk during respiratory virus infection.

POSTER 20 – Sex-Specific Mitochondrial Response may be Protective in the Hyperacute Phase of Neonatal Sepsis

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Introduction: Late-onset sepsis (LOS) is defined as the dysregulated host response to an infection after 72h of life. Metabolic disturbance is a common manifestation of LOS. At the center of metabolic homeostasis, the mitochondrion is a critical hub for energy production and signaling via the generation of reactive oxygen species (ROS). Investigating the mitochondrial response to LOS has yet to be explored. We have previously observed increased plasma aminotransferase activity in septic pups, suggesting hepatocellular damage or altered liver function. Here, we sought to explore the acute effects of LOS on liver mitochondrial function.

Methods: Three-day-old Sprague Dawley pups were injected fecal slurry (FS, 1.0 mg/g body weight) intraperitoneally; controls received vehicle (5% dextrose in water). Pups were euthanized at 4h, 8h, and 24h post-FS. The liver was homogenized for high-resolution respirometry. Remaining tissues were flash frozen for RT-qPCR and Western blotting.

Results: FS reduced pup bodyweight by 4% ($P<0.0001$) by 24h but did not affect absolute male liver weights. Blood glucose rose 4-fold at both 4h and 8h and normalized by 24h. Mitochondrial NADH pathway respiration increased by 62% ($P=0.004$) in male pups at 4h. This was accompanied by 1.8-fold upregulation of total AMPK ($P=0.02$) and 30% increase in phospho-AMPK/AMPK ($P=0.03$) in males. Enhanced NADH pathway respiration was observed at 8h in both sexes ($P<0.0001$), but increased AMPK protein expression was only observed in males with no changes to phospho-AMPK/AMPK. Succinate pathway respiration was enhanced by 65% ($P=0.005$) in male pups at 4h and continued through 8h. By 24h post-FS, neither NADH nor succinate pathway respiration differed from controls. However, total AMPK remained upregulated by 2.7-fold ($P<0.0001$) in both sexes. mRNA expression of H_2O_2-producing gene (Sod2) was upregulated 10-fold ($P<0.0001$) while H_2O_2-removing genes (Cat, Gpx1, Gsr) were downregulated in septic pups by 24h.

Conclusion: Sex-dependent increase in mitochondrial respiration was associated with increased total AMPK at 4h and 8h and increased AMPK activation at 4h, suggesting an adaptive mechanism to supply energy and metabolites in response to infection. However, increased respiration and downregulation of antioxidants may suggest an accumulation of H_2O_2 which could damage cellular integrity.

POSTER 21 – Investigating the mechanism of myeloperoxidase-mediated toxicity of the industrial contaminant, 6-PPD, in vitro

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Purpose: A product of the industrial contaminant, 6-PPD (N-[1,3-Dimethylbutyl]-N'-phenyl-p-phenylenediamine), has been recently reported as highly toxic to coho salmon and potentially toxic to other aquatic organisms. The potential toxicity of 6-PPD in humans, however remains unknown. The enzyme myeloperoxidase (MPO) in neutrophils, is known to produce free radicals and oxidized metabolites from xenobiotics and so its role in 6-PPD-mediated toxicity was investigated.

Methods: UV-visible spectroscopy and liquid chromatography-mass spectrometry (LC/MS) were performed to investigate the MPO mediated oxidation of 6-PPD and identify possible metabolites in the absence/presence of glutathione (GSH). 6-PPD's cytotoxicity, effect on mitochondrial membrane potential (MMP), and GSH depleting ability in the HL-60 cell line, a MPO-rich human leukemia cell line, were assessed. Electron paramagnetic resonance (EPR) and DMPO spin-trap was applied to capture the possible radical products in the presence of GSH.

Results: UV-Vis analysis of MPO-catalyzed oxidation of 6-PPD demonstrated changes in 6-PPD spectrum, whereas GSH addition altered the spectrum indicating possible GSH conjugate formation. LC/MS showed the formation of multiple products, including GSH-6-PPD conjugates, and a GSH conjugate to a 4-hydroxydiphenylamine (a known 6-PPD degradant) which could potentially induce cytotoxicity. 6-PPD demonstrates a positive concentration dependent relationship with cytotoxicity whereas GSH levels was decreased by 6-PPD in HL-60 cells. With increasing 6-PPD levels, MMP levels decreased which suggest cellular mitochondria depolarization occurred. EPR spin-trapping demonstrated a concentration-dependent relationship between 6-PPD and GS radical signal intensity in the presence of H_2O_2. Furthermore, spin-trapping of mitochondrial radical also shows a positive relationship with 6-PPD's concentration.

Conclusion: 6-PPD's oxidation can induce radical products formation and GSH conjugation in the presence of MPO. Furthermore, 6-PPD induces toxicity, disrupts MMP and depletes GSH in MPO-rich HL-60 cells. Our results suggest that MPO could be an activator of 6-PPD's toxicity in humans. A potential relationship between 6-PPD's oxidative metabolites and toxicity mechanism requires deeper investigation to determine its toxicity in mammals, including humans.

POSTER 22 – Cytosolic citrate is needed for nitric oxide production during human sperm capacitation

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Infertility is rising worldwide, as the prevalence of Canadian couples with infertility has increased from 8% in the 1980s to about 17% today. Nearly half of these cases being traced to men, 30% of whom suffer from idiopathic infertility. Thus, there is an unmet need to characterize the mechanisms underlying male infertility.

To recognize and fertilize the oocyte, the spermatozoon must undergo the process of capacitation, which involves a series of biochemical and morphological changes. For capacitation to occur, human spermatozoa require low ROS levels, activation of phosphorylation pathways, and sufficient levels of energy metabolites such as glucose, pyruvate, and lactate.

Citrate is abundant in seminal plasma, with low levels having been reported in idiopathic infertile men. In somatic cells, citrate can be exported from the mitochondrion to the cytosol via the mitochondrial citrate transport protein (CTP). Citrate can then be used by the cytosolic ATP-citrate lyase (ACLY) to produce acetyl CoA and oxaloacetate, which is converted to malate and NAD^+ via malate dehydrogenase. Malate can then be converted to pyruvate and NADPH by the malic enzyme. However, the specific role of citrate in sperm capacitation is unknown.

We hypothesize that citrate is required for energy production and NO• production during sperm capacitation. We determined whether 1) citrate supports sperm capacitation, 2) cytosolic ACLY and mitochondrial CTP are involved in capacitation, and 3) citrate promotes NO• production during capacitation.

Spermatozoa incubated in BWW medium without glucose, pyruvate or lactate, but containing citrate and fetal cord serum ultrafiltrate (FCSu, a capacitation inducer), underwent sperm capacitation at levels similar to those incubated in BWW medium containing energy substrates. In addition, ACLY and CTP inhibition prevented FCSu-induced capacitation in spermatozoa incubated in citrate-containing BWW medium, in presence or absence of glucose, pyruvate and lactate. In these same conditions, L-NAME, an inhibitor of nitric oxide synthase, prevented capacitation, suggesting the need of citrate during capacitation for NO• production.

In conclusion, cytosolic citrate supports sperm capacitation by a mechanism promoting energy substrates (e.g. pyruvate) and NO• production. This research will help understand citrate regulation in sperm capacitation and will ameliorate treatment strategies for male infertility.

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POSTER 23 – Blood Ascorbic acid levels correlate with specific immune system proteins in subjects with hyperinsulinemia

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Background: Vitamin C (ascorbic acid) is an important water-soluble antioxidant associated with decreased oxidative stress in type 2 diabetes patients. A previous targeted serum proteomic study has indicated that ascorbic acid is associated with markers of the immune system. Studies also have reported that a high intake of dairy products may modify the risk of type 2 diabetes. However, it is unknown whether high dairy intake modifies the association of serum ascorbic acid with specific proteins.

Objective: To identify serum proteins that correlate with serum ascorbic acid levels and uncover a predictive protein signature for ascorbic acid levels among overweight individuals with hyperinsulinemia.

Methods: In this crossover clinical trial, 25 hyperinsulinemia subjects (54±14 yrs and BMI 31±3) were randomly assigned to high dairy (HD) products intake (≥4 servings/day according to Canada's Food Guide (2007)) or adequate dairy (AD) product intake (≤2 servings/day) for 6 weeks, then crossed-over after a 6-week washout period. Serum levels of ascorbic acid were measured using ultra performance liquid chromatography-mass spectrometry. Liquid chromatography-mass spectrometry was used to identify and quantify 231 serum proteins. Spearman correlation followed by gene ontology analyses were performed to identify biological pathways associated with ascorbic acid. Finally, machine learning analysis was performed to obtain a specific serum protein signature that could predict ascorbic acid levels.

Results: After adjustments for waist circumference, LDL, HDL, fasting insulin, fasting blood glucose, age, gender, and dairy intake; serum ascorbic acid correlated positively with different aspects of the immune system. Machine learning analysis indicated that a signature composed of 21 features that included 17 proteins (mainly from the immune system), age, sex, waist circumference, and LDL could predict serum ascorbic acid levels in hyperinsulinemia subjects. Dairy product intake was not part of this signature.

Conclusion: Serum levels of ascorbic acid were correlated with proteins that regulate different aspects of the immune response. Finally, high dairy intake does not predict serum ascorbic acid levels in hyperinsulinemia subjects.

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POSTER 24 –The oxidative-stress sensor TRPM2 in TNBC

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Relevance- Despite medical advances, breast cancer (BC) remains the second leading cause of cancer death in women worldwide. The triple-negative breast cancer (TNBC) subtype has the worst prognosis and the highest mortality rate. Worse, once a tumor becomes chemoresistant, there is no treatment. Therefore, investments in research that provide new insights into the pathology of TNBC and new therapies are a high priority to reduce the negative impact of BC. Background-Cancer cells exhibit elevated levels of reactive oxygen species (ROS), which exacerbate the malignant phenotype by maintaining and reinvigorating cell proliferation and metastasis. While the exact mechanism by which ROS drives cancer progression is still largely unknown, there is increasing evidence that the TRPM2 channel, the major oxidative stress sensor and calcium permeable, is involved. Our hypothesis-Elevated TNBC ROS activates TRPM2-mediated calcium influx, which 1) supplies mitochondrial calcium uptake and controls mitochondrial structure function, thereby promoting TNBC progression, and 2) activates mitophagy, likely by modulating AMPK-mTOR-dependent signaling, thereby maintaining mitochondrial quality and reducing ROS production. Results-We used MDA-MB -231 as a model for TNBC and MCF10A as noncancer cells. We found that 1) TRPM2 expression negatively correlates with BC patient survival and is highly expressed in TNBC cells compared to MCF10A cells; 2) downregulation of TRPM2 reduces cell proliferation, induces G0/G1 cell cycle arrest and apoptosis; 3) silencing of TRPM2 inhibited cell migration and xenograft growth; 4) knockdown of TRPM2 inhibited mitochondrial calcium uniporter expression, reduced mitochondrial calcium uptake capacity, altered mitochondrial electron transport chain complexes, and reduced ATP production. 5) Suppression of TRPM2 inhibited mitophagy and increased the production of ROS and NO. 6) Inhibition of TRPM2 improved the efficacy of doxorubicin. CONCLUSION: Our data suggest a key role for the oxidative stress sensor TRPM2 in TNBC survival through its role in mitochondrial fuel supply processes to meet increased metabolic demands for growth and proliferation and through its role in mitophagy to buffer production of ROS to maintain efficacy.

POSTER 25 – Harnessing oxidative stress imbalance to boost light-mediated therapies against pathogens

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Each year a rising number of infections can not be successfully treated owing to the increasing pandemic of antibiotic-resistant pathogens. The global shortage of innovative antibiotics fuels the emergence and spread of drug-resistant microbes. Basic research, development, and applications of alternative therapies are urgently needed. Since the 90´s, light-mediated therapies have promised to be the next frontier in combating tenacious microbes. These platforms have demonstrated to be a reliable, rapid, and efficient alternative to kill pathogens while avoiding the emergence of resistance mechanisms.

In this presentation, I will focus on how to exploit photodynamic inactivation (PDI) to treat a wide range spectrum of infectious diseases. Especially, I will shed light on novel photosensitizer systems (PSs) and protocols for the detection and/or generation of reactive oxygen species. Results evidence the outstanding killing efficiency of the PSs in vitro, and in vivo against clinical isolates of ESKAPE pathogens with excellent biocompatibility. Furthermore, ex vivo studies on a skin model reveal that phototherapy can be used as a prophylaxis method to control animal infections. Practical application in the elimination of resilient photogenic microbes and study of photooxidation mechanisms will be highlighted.

Our endeavor is to push the limits of PDI to a fundamental understanding of the overall effect of the therapy at the single-cell level and all the way to assemble cellular communities of these pathogenic species.

POSTER 26 – Semi-targeted LC-HRMS/MS analysis of bile acids to decipher the effects of drug-induced liver injury in humans

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Acetaminophen (APAP) is a very common analgesic used worldwide to relieve pain and decrease fever. Unfortunately, when taken excessively, this drug can cause acute liver failure, through the formation of its reactive metabolite, N-acetyl p-benzoquinone imine (NAPQI). This reactive species binds to liver proteins leading to liver failure. This affects hundreds of thousands of people each year in North America and constitutes the main cause of acute liver failure in the Western world. Previous studies have shown that APAP can influence the level of circulating bile acids by disrupting the blood-bile barrier. Increased levels of bile acids is a marker of liver disease.

To investigate the effects of acetaminophen on circulating bile acid profiles, metabolites were extracted from human serum samples from patients with APAP-related acute liver failure and healthy controls. Bile acids were analysed by LC-MS/MS and all peaks detectable in samples from a standard mix of 46 bile acids were assigned based on accurate mass and retention time. Then, potential bile acid isomers not contained in the standard mix, as well as putative glucuronide and sulfate conjugates of these bile acids were assessed by accurate mass filtering. Our results showed that acetaminophen significantly influenced the levels of bile acids and conjugates. Several peaks varied significantly between healthy controls and ALF patients, as well as some statistically relevant changes based on patient outcome.

POSTER 27 – Early-life exposure to parenteral nutrition induced-peroxides changes DNA methylation and expression of antioxidants genes in guinea pigs

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Background: Neonatal parenteral nutrition (PN) is contaminated with peroxides. PN oxidizes the redox potential of glutathione, which induces global DNA hypermethylation. Because oxidative stress influences several metabolic diseases observed in adults, we suspect that PN-induced oxidative stress during neonatal period leads to epigenetic modulation of genes encoding glutathione metabolism and that these modifications persist over time.
Objective: To assess the hepatic impacts of a neonatal PN enriched or not in glutathione, as an anti-peroxide, on the methylation and expression of the NRF2, GCLC, GSS, GPx1 genes in animals that received a 4-days PN during their first week of live and 15 weeks after stopping PN.
Methods: 3-day-old guinea pigs randomized to receive a standard diet (Control group), PN or PN+GSSG for 4 days. At 1 week of life, half of them were sacrificed whereas all others received standard diet over 15 weeks. Determinations: hepatic DNA methylation was analyzed by Methylated DNA immunoprecipitation (MeDIP)-qPCR and relative gene expression by RT-qPCR of redox sensitive transcription factor (NRF2) and genes involved in glutathione pathways (GCLC, GSS, GPx1). Statistics: ANOVA, $p<0.05$.
Results: For GPx1, compared to control, we observed an increase in DNA methylation and lower mRNA expression only in PN group regardless of age. Regarding NRF2, there was no difference between control and PN, while there was lower DNA methylation and higher level of mRNA in the PN+GSSG group, independently of age. Compared to control, PN induced no DNA methylation changes of GCLC and GSS but a down-regulation of their mRNA expression, irrespective of age. These changes of PN were prevented by glutathione (GSSG) supplementation, in both neonatal and adult animals.
Conclusion: In the concept that inadequate nutrition in early life can influence disease risk lifelong, the results suggest that the risk may be caused by oxidative stress such as that induced by PN. The similarity of the results observed during the treatments and those observed 15 weeks after stopping those treatments suggests a persistent imprint over time. Because most of these observations were avoided by adding glutathione (GSSG) in PN, this suggests that oxidative stress is the triggering effect.

POSTER 28 – Oxidising and antiproliferation properties of half-sandwich Os(II) complexes with indomethacin-functionalized ligand

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Although platinum-based anticancer drugs have an indisputable position in cancer therapy, chemists are motivated to search for new metal-based therapeutics due to their side effects and resistance. Osmium compounds have been shown to be suitable anticancer agents with significant anticancer activity in vitro and in vivo, acting through a non-conventional redox-mediated mechanism of action. In this work, organic compound 5-(pyridin-2-yl)-1,3,4-thiadiazol-2-amine (L1) and its derivatives L2 and L3 were prepared and used for reactions with dinuclear Os (II) intermediate to form three new half-sandwich complexes (1-3). Two bear the COX inhibitor indomethacin as a bioactive substituent bound through the enzymatically cleavable amide bond (2) or the aliphatic linker (3). Anticancer activity of various half-sandwich complexes (e.g.Ru or Ir) is linked with glutathione metabolism. In our experiments, complexes 1, 2 and 3 converted GSH to its oxidised form GSSG. The oxidation efficiency towards the GSH oxidation was markedly higher for complex 3. Similarly, complexes 1-3 oxidised NADH to NAD+. In this case, the efficiency was comparable for 1 and 3 but markedly lower than for 2. The antiproliferative effect of complexes was evaluated at ten cell lines (nine cancer ones and MRC-5 lung fibroblasts) exposed to 10 μM substance for 48 h, and the changes in cell proliferation were compared. The unsubstituted complex 1 did not affect cancer cell proliferation. In contrast, the indomethacin-substituted complexes 2 and 3 reduced the cancer cell proliferation at some of the used cell lines (HT-29 and HeLa cells for 2, and A2780, HeLa and MCF-7 for 3). To conclude, complexes 1, 2 and 3 effectively oxidised the reduced GSH and NADH and thus represent the first half-sandwich Os(II) GSH oxidisers. The best-performing complex 3 was more potent in decreasing the proliferation of the MCF-7 breast carcinoma (IC50 = 8.2 μM) compared with non-cancerous MRC-5 fibroblasts (IC50 = 18.2 μM). This study was supported by the InoMed project (Reg. No.CZ.02.1.01/0.0/0.0/18_069/0010046) co-funded by the European Union.

POSTER 29 – Newly Discovered Sulfenylation of STAT1 Controls IFNg-Induced Gene Regulation

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The STAT1 transcription factor is a pivotal regulator of gene expression induced by different cytokines, including Interferon g (IFN g), in processes such as antiviral and antibacterial defense, immune tolerance, suppression of cell proliferation, and induction of apoptosis. STAT1 activation state is finely tuned by different Post Translational Modifications (PTMs) types. These PTMs play a crucial role in determining the intensity and duration of STAT1 activity and, thus, the biological response it elicits. Reactive oxygen species regulate cell signaling through reversible oxidative post-translational modifications (ox-PTMs) of Cys residues in proteins. Here, we investigated the role of reversible Cys ox-PTMs in STAT1 function. Using maleimide-derivative bioswitch methods to label Cys ox-PTMs and immunoblotting, we have demonstrated that both diamide, a thiol-oxidizing agent, and HOCl, resulting from myeloperoxidase activity, induce reversible ox-PTMs of STAT1 Cys in various cell types. Furthermore, using DCP-Bio1 Labeling, we observed STAT1 sulfenylation in response to oxidants and IFNg stimulation. Analysis of Cys/Ala mutants of STAT1 revealed gain-of-function phenotypes of mutations in two different functional domains associated with increased phosphorylation, nuclear localization and gene expression. Accordingly, the oxidation of STAT1 by oxidants impaired STAT1 phosphorylation. Collectively, our data support a model in which previously unrecognized ox-PTMs of STAT1 Cys residues serve as an essential regulator of STAT1 activity. Ongoing studies aim to elucidate STAT1 oxidation sites by further proteomics analysis.

POSTER 30 – The impacts of cannabidiol (CBD) on mitochondrial function and redox homeostasis of trophoblast stem cells in vitro

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About 2-28% of pregnant women have reported using cannabis for nausea and relief of common pregnancy symptoms (Corsi et al. 2019, 2020); however, emerging evidence suggests that the use of cannabis during pregnancy is correlated to dysfunctional placentation and poor pregnancy outcomes, such as intrauterine growth restriction. Placentation occurs through the differentiation of trophoblast stem cells, or cytotrophoblasts (CTBs), into a multinucleated syncytiotrophoblast (STB), which comprises the maternal-foetal interface. A major component of cannabis, cannabidiol (CBD), has been demonstrated to cross the placental barrier and disrupt mitochondrial homeostasis in vivo. Previous work from our group showed that delta-9-tetrahydrocannabinol (D9-THC) treatment induced mitochondrial dysfunction, as evidenced by diminished mitochondrial respiration, and upregulated heat shock proteins and antioxidant scavengers in BeWo cells. Considering the well-established pharmacological similarities between D9-THC and CBD, we sought to characterize the impacts of CBD on mitochondrial function and redox homeostasis in BeWo b30 trophoblast cells. We hypothesize that CBD alters the antioxidant profiles and diminishes mitochondrial respiration in CTBs and STB. Using MTT and LDH assays, we determined that [CBD]>30μM were cytotoxic to BeWo b30 cells. Treatment with 20μM CBD increased mRNA levels of Hsp70 4-fold and significantly downregulated Sod1 in CTBs. In contrast, differentiated STB cells observed a 0.5-fold increase in Hsp60 and decreases in both Hsp70 and Sod1 mRNA levels following treatment with 20μM CBD. Levels of reactive oxygen species (ROS) were significantly increased in STB, but not CTB, following 20μM CBD treatment. In addition, mitochondrial respiration was found to be diminished in both CTBs and STB. Overall, CBD alters the antioxidant activity and impairs mitochondrial function in trophoblasts; however, the impacts of CBD are less pronounced than D9-THC, and in some cases, exhibit opposite trends. In fact, our lab has demonstrated that 20μM of D9-THC upregulated mRNA levels of Hsp60, Hsp70, Sod1 and Sod2 in STB, and that levels of ROS were upregulated with 20μM of D9-THC in STB, highlighting the similarities and differences between CBD and D9-THC (Walker et al. 2020). Our findings suggest that D9-THC and CBD are capable of altering redox homeostasis and inducing mitochondrial dysfunction, which could ultimately lead to oxidative stress and dysfunctional placentation.

POSTER 31 – Targeting redox homeostasis to induce DNA damage and an inflammatory phenotype in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease that remains resistant to all treatments. This resistance is mostly driven by the presence of immune inhibiting cell populations in a physically dense fibrotic tumour microenvironment (TME) that shield PDAC from conventional antitumor therapies. Interestingly however, PDAC cells are surrounded by a TME already infiltrated with effector T-cells, only requiring activation. Our current work aims to reactivate the suppressive immune infiltrate using disruption of redox homeostasis to improve PDAC sensitivity to existing therapies, including immunotherapy (IT).

DNA damage and cell death are potent activators of innate immunity signaling. Our lab has shown that targeting peroxiredoxin 4 (PRDX4), which metabolizes hydrogen peroxide in the endoplasmic reticulum, results in high levels of reactive oxygen species (ROS), DNA damage, cancer cell death, and increased survival of tumor-bearing mice. Here we aim to investigate whether the accumulation of DNA damage resulting from PRDX4 loss triggers inflammatory pathways that could result in immune cell activation, through the innate ability of cells to sense cytosolic DNA. Our work to date has mostly focused on the STING pathway, shown to directly link cytosolic DNA sensing from DNA damage to transcription of inflammatory genes, including proinflammatory cytokines and chemokines.

To address this research question, we used genetically engineered human PANC-1 PDAC cells expressing doxycycline (DOX)-inducible shRNA targeting PRDX4. We observed an upregulation of a subset of inflammatory genes upon PRDX4 knockdown (KD), which is rescued upon siRNA mediated STING KD and STING activity inhibition, demonstrating the involvement of STING upon PRDX4 loss. Furthermore, probing for the presence of cytosolic DNA by immunofluorescence (IF) revealed substantial accumulation of both micronuclei and free cytosolic DNA upon PRDX4 loss. These results suggest that PRDX4 targeting induces ROS production that is associated with release of cytosolic DNA and STING-mediated inflammatory gene activation in cells. Our next steps now aim to identify the cytosolic DNA sensor(s) involved in mediating this inflammatory gene activation but also to assess how the ROS-driven cytosolic DNA drives this phenotype, to leverage it in vivo, in combination with IT.

POSTER 32 – Impact of PERK overexpression in response to severe heat stress

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When exposed to thermal stress, cells activate various protective mechanisms to maintain cellular homeostasis. One such mechanism is the activation of Protein kinase R-like ER kinase (PERK). PERK is a transmembrane receptor of the endoplasmic reticulum (ER) that is involved in the regulation of the cellular stress response to various types of stress. PERK initiates a signaling pathway that leads to the regulation of genes involved in the antioxidant response via Nuclear factor erythroid 2-related factor 2 (Nrf2). It also suppresses protein synthesis through phospho-eukaryotic initiation factor 2 α (p-eIF2α) to prevent further cellular damage. This study aims to determine if overexpression of PERK could be beneficial and protects cells against severe heat stress. A HeLa (WT) cell line that overexpresses PERK (PERK OE) was developed and tested for its ability to protect against severe heat stress at 42°C. Surprisingly, the overexpression of PERK did not confer the expected protective effects. Instead, the PERK overexpressing cells demonstrated an increase in caspase-9, caspase-3, and calpain activities, along with high levels of reactive oxygen species (ROS) compared to the WT, which are all indicative of cellular damage. PERK OE cells showed increased expression of key stress response genes such as perk (EIF2AK3), eIF2α, catalase, ATF4, nrf2 and MnSOD. At the protein level, the cells demonstrated higher expression of PERK, phospho-PERK, phospho-eIF2A, ATF4, BAX, Sestrin-2 (SESN2), and p62 in comparison to the WT. Increased protein expression was maintained over time when cells were heated at 42°C. Many elements could be the cause of this phenomenon. The overexpression of PERK could impact the proliferation rate and induce cell-cycle arrest. Overall, these findings suggest that PERK overexpression may not be beneficial in protecting cells against severe heat stress, however new avenues should be explored.

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POSTER 33 – Sensitivity to Spontaneous Liver Fibrosis is Comparable in C57BL/6 and AKR Mouse Models of Hereditary Hemochromatosis

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Hemochromatosis is a disease of iron overload that is caused by mutations in genes involved in the hepcidin BMP/SMAD signalling pathway. The role of hepcidin is to bind and degrade the iron exporter ferroportin. Therefore, when hepcidin transcription is inactivated, iron export from cells goes unchecked which eventually causes systemic iron overload. Mouse models of hemochromatosis recapitulate iron overload but do not develop the clinical complications observed in humans. This also applies to hemojuvelin-deficient (Hjv-/-) mice, which represent a model of early-onset juvenile hemochromatosis with more prominent iron overload compared to other genetic types. These mice are deficient in the hemojuvelin (Hjv) enzyme, a BMP co-receptor that is crucial for the transcription of hepcidin.

Our laboratory and others showed that Hjv-/- mice in the C57BL/6 background do not develop spontaneous early liver disease. Nevertheless, another model of juvenile hemochromatosis (double HFE and TfR2 knockout mice) in the AKR background was shown to develop spontaneous liver fibrosis. Notably, humans with double HFE and TfR2 inactivation exhibit the same clinical phenotype as those with HJV deficiency. Thus, we hypothesized that Hjv-/- mice in AKR background would be sensitized to spontaneous liver fibrosis, and that the genetic background is a critical determinant of sensitivity to liver disease.

To address this hypothesis, we backcrossed our Hjv-/- mice from C57BL/6 to the AKR background for 10 generations. We then established that Hjv-/- mice in C57BL/6 and AKR background exhibit a similar degree of iron overload. Serum markers of liver injury (ALT, AST) were also comparable for both backgrounds, although slightly higher in AKR mice. Preliminary liver histological analysis did not reveal signs of spontaneous fibrosis in Hjv-/- mice of either C57BL/6 or AKR background at the age of 15 weeks. Additional experiments are underway to investigate potential phenotypic differences between C57BL/6 and AKR Hjv-/- mice at older age.

POSTER 34 – Targeting the Cell Surface GRP78 (csGRP78) and Anti-GRP78 autoantibodies complex in association with inflammation activity in atherosclerosis

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Background/Objectives: Cardiovascular diseases are major causes of mortality worldwide. Most heart attacks are a result of atherothrombosis, which is a process of thrombus formation superimposed on disrupted atherosclerotic plaques. During early stages of atherogenesis, endothelial cells (ECs) can suffer from oxidative stress resulting in increased levels of proinflammatory factors in the vessel walls, and accumulation of macrophage foam cells. Macrophages can secrete cytokines, which enhance reactive oxygen species (ROS). ROS can lead to smooth muscle cell migration and stimulate MMP secretion leading to plaque instability. We have shown that enoxaparin, a low molecular weight heparin, inhibits the interaction between csGRP78, a molecular chaperone, and anti-GRP78 autoantibodies resulting in a decrease in inflammation and plaque development. However, enoxaparin poses a bleeding risk. Given this limitation, we screened a library of potential GRP78 small chemical compounds (referred to as GRP78 binders) for their ability to modulate binding and inflammation activity in the presence of autoantibodies.

Objective: To determine if these GRP78 binders: 1) inhibit the interaction between GRP78 and the autoantibodies, and 2) mitigate autoantibody induced inflammation activity in ECs

Methods: To test if the GRP78 binders disrupt the binding between the GRP78/autoantibody complex a GRP78 ELISA is used. Recombinant human GRP78 is plated, GRP78 binders are treated for 24 hours before incubating with the autoantibodies. The plate is then washed and incubated with an anti-human HRP antibody for 1 hour. Next, immunoblotting is conducted with a similar treatment strategy to analyze inflammation activity.

Results: 100 GRP78 binders were screened using the GRP78 ELISA to see if they decrease the interaction between GRP78 and the autoantibodies. Through this screen one compound, B7, caused a consistent decrease in the interaction between GRP78 and the autoantibodies. B7 was next tested for NF-κB inhibition through blotting and there was significant decrease in phosphorylated p65 and IκBα levels.

Conclusions: Our findings show that B7 inhibits the interaction between recombinant human GRP78 and autoantibodies as well as inhibits inflammatory activity, as determined by NF-κB activation. We are further testing B7 for its ability to mitigate inflammatory effects in ECs and in mouse models for lesion development.

POSTER 35 – CONFIDENCE: An App for Cross-Platform Differential Gene Expression Analysis and its Use in Studies of Metabolism and Oxidative Stress

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An increasing emphasis has been placed on the role of dysregulated gene expression and abnormal transcriptional patterns as markers of cardiovascular and metabolic disease. Numerous platforms have been developed for differential gene expression analysis on data from Next-Generation RNA Sequencing. These platforms utilize substantially distinct statistical models and normalization processes to evaluate genes that are significantly differentially expressed. However, the use of these platforms and the interpretation of results rely on substantial programming knowledge and proficiency; therefore, investigators without a bioinformatics background studying the transcriptional drivers of metabolic diseases are often unable to explore this omics discipline. The implementation of a simple, user-friendly program that provides such an analysis across numerous platforms as well as a functional interpretation of these gene expression patterns has yet to be published. Here we present CONFIDENCE, a web-based application aimed to make transcriptomic analyses easy and accessible. CONFIDENCE allows users to input gene count files retrieved from Next-Generation RNA Sequencing and obtain a list of differentially expressed genes, functional pathways, and high-quality figures suitable for publishing. The app utilizes the most frequently used differential expression platforms to provide a confidence score for outputted genes and pathways. The confidence score includes the number of platforms that output a common differentially expressed gene and a custom score incorporating each gene's false discovery rate and Log2(Fold Change). Using a publicly available dataset representing the skeletal muscle transcriptome of mice fed either a High Fat Diet or Control, we have outlined numerous differentially expressed genes found across all platforms representing fatty acid metabolism, oxidoreductase, and vitamin binding pathways. These genes encode for Fasn (fatty acid synthase), Ech1 (Enoyl-CoA Hydratase 1), Acly (ATP Citrate Lyase), and Acaca (Acetyl-CoA carboxylase 1) which were found to be upregulated in High Fat Diet-fed mice. The gene targets obtained from this 'cross-platform' pipeline can be modulated in metabolic studies with greater assurance, which increases the efficiency of result translation. The program also offers numerous options that allow for a broad customization of figure outputs for publishing purposes. To promote the initiative of open science, the CONFIDENCE application will be made publicly available.

POSTER 36 – Elevated serum fibroblast growth factor 23 predicts mortality in HIV/HCV coinfected patients

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Background. Liver disease is a leading cause of mortality in people with hepatitis C virus (HCV) and HIV co-infection.Identifying biomarkers to predict adverse clinical outcomes is pivotal for risk stratification in such high-risk patients. Fibroblast growth factor 23 (FGF23) is a hormone produced primarily by bones that regulates phosphate and vitamin D homeostasis. When elevated, it has been found to predict poor clinical outcomes in patients with chronic kidney disease, heart failure, and chronic liver disease due to a single etiology. To date, FGF23 has not yet been employed in the context of HCV/HIV coinfection.

Aims. 1) To investigate whether elevated FGF23 is associated with all-cause mortality in HCV/HIV coinfected patients; 2) To develop a putative hypothesis for the cause-and-effect relationship between FGF23 as exposure and all-cause mortality as outcomes and estimate the causal mediation effect of advanced liver fibrosis as a mediator.

Methods. Patients from the Canadian Coinfection Cohort who had HCV/HIV coinfection, available serum FGF23, the data needed to calculate fibrosis-4 (FIB-4) score, and at least a year of follow-up were included. Elevated FGF23 and advanced liver fibrosis were defined as a plasma level >241 RU/ml and FIB-4 score >3.25, respectively. All-cause mortality was determined using survival analysis. The effect of advanced liver fibrosis as a mediator on mortality was estimated using mediation analysis and depicted on a directed acyclic graph (DAG).

Results. A total of 321 patients were included (24% with elevated FGF23 and 19% with advanced liver fibrosis). During a median follow-up period of 8.4 years, 34% of the cohort died. Patients with elevated FGF23 had a greater incidence of all-cause mortality (66.1, 95% CI 45.8-92.3/1000 PY) than those with normal FGF23 (37.5, 95% CI 29.6-46.9/1000 PY). After adjusting for potential confounders, elevated FGF23 was associated with significant direct and indirect effects (mediated through advanced liver fibrosis) on all-cause mortality, with 43% of deaths mediated via the mediator.

Conclusions. In HCV/HIV coinfected patients, elevated FGF23 is associated with increased all-cause mortality both through and independently of advanced liver fibrosis. Thus, FGF23 could be used as a prognostic biomarker for risk stratification in this population.

POSTER 37 – Elucidating the role of transcriptional regulator MED15 in cancer cell oxidative stress response

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The aberrant growth, a hallmark of cancer, leads to cancer cells experiencing oxidative stress, i.e., an excess of reactive oxygen species (ROS). In turn, this causes DNA damage and genome instability. To grow and thrive despite oxidative stress, cancer cells activate cellular pathways such as the transcription factor NFE2-related factor 2 (Nrf2), which rewire the transcriptome to promote oxidative stress resistance. Therefore, mapping oxidative stress response pathways is an important area of research that can lead to potential new drugs. Our lab previously studied oxidative stress signaling in C. elegans, which features SKN-1 as the C. elegans homolog of Nrf2. We found that the Mediator complex subunit MDT-15 is an essential cofactor of SKN-1, but the role of the human homolog MED15 is unknown. MED15 is upregulated in many cancer types, and aberrant MED15 activation is important for cancer development, especially in metastasis and treatment resistance. To study the role of MED15 in cancer, we generated three MED15 knockout (KO) cell lines in the A549 lung adenocarcinoma cells using CRISPR-Cas9 genome editing. Transcriptome analysis showed that oxidative response genes were downregulated in these MED15 KO cells, implying that MED15 is vital for the oxidative stress response in lung cancer cells. To further examine how MED15 affects oxidative stress response, I treated MED15 parental and KO cells with different oxidative stress inducers such as tert-butyl hydroquinone (tBHQ), sulforaphane, and transforming growth factor β (TGF-β). I then conducted qPCR and western blot to determine changes in oxidative stress response genes such as heme-oxygenase 1 (HO-1). The results showed that induction of HO-1 was inhibited in MED15 KO cells, suggesting that MED15 KO leads to susceptibility to oxidative stress. To further understand the molecular mechanism, I will conduct co-immunoprecipitation (co-IP) to determine if MED15 protein binds to Nrf2, and chromatin immunoprecipitation (ChIP) to determine if MED15 binds to Nrf2 antioxidant response elements in target gene promoters. Understanding the role of MED15 has clinical potential, as MED15 can be targeted by small molecules, making it a promising drug target, and it can uncover a new potential pathway in cancer growth and resistance.

POSTER 38 – Constant alcohol consumption exacerbates neurological decline and causes neuronal cell loss in rats with chronic liver disease

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Background: Hepatic encephalopathy (HE) is a debilitating neurological complication of chronic liver disease with alcohol being a common etiological factor. However, excessive alcohol consumption has been shown to impact neurological integrity. To date, the influence of alcohol in the development of HE remains unclear. Therefore, we examined the effect of constant alcohol consumption on neurological decline in rats with chronic liver disease induced via bile-duct ligation (BDL).

Method: 5-week BDL rats and Sham-operated controls were used. Day 7 after surgery, rats were administered alcohol twice a day (dose of 3g/kg, via gavage) for 4 weeks. Motor coordination (rotarod) and anxiety-like behavior (open field (OF) and elevated plus maze (EPM)) were assessed. Upon sacrifice, brains were collected, and western blot and immunohistochemical (IHC) analyses were used to investigate neuronal integrity in frontal cortex and cerebellum.

Results: Alcohol (BDL-alcohol rats) further impaired motor coordination at weeks 2, 3, 4, and 5 compared to SHAM-alcohol ($p<0.01$). Furthermore, BDL-alcohol rats demonstrated an increase in anxiety-like behavior; increase in time spent in the closed arms of EPM and decrease in time spent in the center of the OF ($p<0.05$ vs SHAM-alcohol). BDL-alcohol rats demonstrated a decrease in neuronal markers of NeuN and SMI311 ($p<0.01$ and $p<0.05$, respectively), an increase in apoptotic markers of cleaved/pro-caspase3 ($p<0.001$), an increase in necroptosis markers of pRIP3 and pMLKL ($p<0.01$ and $p<0.001$, respectively), a decrease in total antioxidant capacity ($p<0.001$) and an increase in oxidative stress marker of 4-HNE ($p<0.05$) in the cerebellum (not found in frontal cortex) compared to all groups. IHC results confirmed the colocalization of apoptotic marker (cleaved Caspase3) and necroptosis marker (pMLKL) in the granular and Purkinje layer neurons of the cerebellum of BDL-alcohol rats.

Conclusion: Constant alcohol consumption exacerbates HE and leads to neuronal loss via apoptosis and necroptosis in the cerebellum. Additionally, higher levels of oxidative stress marker of 4-HNE and decreased total antioxidant capacity in the cerebellum of BDL-alcohol rats suggest that oxidative stress is a triggering factor leading to neuronal loss/injury. These results demonstrate an adverse effect of constant alcohol consumption on the development of HE and neuronal integrity in chronic liver disease.

POSTER 39 – Time-dependent changes to skeletal muscle macrophage redox homeostasis in Myositis

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Introduction: Myositis is a rare autoimmune mediated disease, resulting in muscle weakness and dysfunction. While myositis has been associated with an increase in muscle fibre mitochondrial reactive oxygen species H2O2 emission, the role of this stress response in muscle-resident macrophages (a prominent immune cell) has yet to be explored. The purpose of this research is to elucidate the muscle and time dependent relationship between myositis-induced myopathy and changes in macrophage mitochondrial superoxide production and total cellular reduced glutathione.

Methods: 12-16 week old female BALB/c mice received multiple injections with rabbit skeletal muscle-derived myosin and were assessed at 21, 28 and 49 days (d) post final injection as follows: 21d, 3 injections at day 0, 7, 14; 28d and 49d, 4 injections at day 0, 7, 14, 21. Control groups were comprised of either a vehicle (glycerol+saline- GS), or a vehicle and an adjuvant (glycerol+Complete/Incomplete Freund's Adjuvant (CFA/IFA) - GCI). The experimental group (M) received rabbit-derived skeletal muscle myosin emulsified with CFA/IFA to induce a model of experimental autoimmune myositis (EAM). These adjuvants were used to stimulate a stronger immune response to the myosin antigen. Following tissue collection, flow cytometry was used to assess macrophage content and characteristics (superoxide production and total glutathione content) in two hindlimb muscles.

Results: At 49d, resident macrophages from gastrocnemius demonstrated a lower proportion of monobromobimane (mBBR; reduced glutathione tag) positive cells in GCI and M relative to GS (-47.5% and -40.8%, respectively, $p<0.05$) with no differences between GCI and M. There were no differences in MitoSOX fluorescence (superoxide probe) between any groups. In soleus, no differences in mBBr or MitoSox were detected between any groups, nor were there differences at 21d and 28d in either muscle. Analysis of muscle and organ wet weights did not reveal significant differences between the control and experimental groups.

Conclusion: The findings suggest that the reduction in GSH-positive cells is due more to adjuvant-induced inflammation rather than a response to myosin itself. Remaining analyses will relate these findings to the time-dependent and muscle-specific force and histological responses to the adjuvant and myosin antigen.

POSTER 40 – Impact of Covid-19 Antiviral Drugs on Neutrophil Oxidative Activity

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Purpose: The Coronavirus disease (COVID-19) pandemic affected millions of lives worldwide and continues to cause morbidity. The purpose of this study was to investigate the novel activity of SARS-CoV-2 treatment drugs and their metabolites in modulating neutrophil function as neutrophils are important players in COVID-19 patient outcomes. Hypothesis: Based on chemical structure, we hypothesized that COVID-19 drugs will be oxidized by neutrophil myeloperoxidase (MPO). Objectives: a) investigate if COVID-19 drugs can be enzymatically metabolized by MPO, and identify if they form free radical metabolites, and b) determine the effect of COVID-19 drugs on human neutrophil free radical formation. Methods: The COVID-19 antiviral drugs remdesivir, molnupiravir, favipiravir and their derivatives were studied by the electron paramagnetic resonance (EPR) spin trapping technique with DMPO (5,5-dimethyl-1-pyrroline N-oxide) to identify and analyze the drug free radical metabolites or glutathione (GSH) free radicals (DMPO/$^{\cdot}$SG). The experiments were designed for enzymatic (MPO) and cellular studies using neutrophils from healthy donors (activated by phorbol myristate acetate). LC/MS was used to analyze drug remaining. Results: MPO experiments showed that remdesivir derivatives produced weakly detectable free radical metabolites compared to structurally related adenosine, which was the most intense. Favipiravir and molnupiravir also formed apparent free radical metabolites. Preliminary results by LC/MS revealed that 47% of molnupiravir was metabolized by MPO, but for remdesivir only 6% was metabolized. GSH oxidation in this system, however, did not show DMPO/$^{\cdot}$SG for remdesivir derivatives and favipiravir. However, molnupiravir attenuated DMPO/$^{\cdot}$SG formation. Activated neutrophils treated with molnupiravir showed stabilized DMPO/$^{\cdot}$OOH (superoxide) formation, and no free radical drug metabolite was detected with superoxide dismutase. (Other drugs are currently under study). Conclusion: This is the first report on the interplay of COVID-19 drugs with neutrophils and specifically neutrophil MPO. From the tested drugs, molnupiravir appeared to impart unique inhibitory activity on MPO and the neutrophil respiratory burst, which requires further investigation. MPO inhibition is a relevant therapeutic area, and there are MPO inhibitors in clinical trials for cardiovascular diseases. Our findings reflect an additional mechanism of action for these drugs, and potentially lead to focusing on MPO inhibitors for treatment of COVID-19.

POSTER 41 – Cre-dependent Sod1 knockout in mouse embryonic fibroblasts

Long Truong-Ong[1], Ying Wang[1], Siegfried Hekimi[1]

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Sod1 codes for a superoxide dismutase, an important enzyme for converting superoxide into hydrogen peroxide, both of which are reactive oxygen species. In humans, this enzyme's activity is associated with many diseases such as Down's syndrome, Parkinson's disease, cancer, and familial amyotrophic lateral sclerosis. Although mice without the Sod1 gene display accelerated muscle atrophy with age, a reduction of ~30% in lifespan, and an increased incidence of liver cancer, they remain viable. Considering how highly Sod1 is conserved between species, the importance of its function, and its high levels of expression, the viability of these mice is surprising. However, the same cannot be said for the mouse embryonic fibroblasts (MEFs) collected from mice without the Sod1 gene. MEFs without Sod1 die after a few days in cell culture. This contradiction between the viability of the mice and the poor survival of their MEFs is not understood. Our hypothesis is that after knocking out Sod1, some aspect of the increased superoxide levels, or the lowered nitric oxide (that is expected to result from high superoxide), or changes in hydrogen peroxide levels, or a combination of these factors, lead to the death of MEFs lacking Sod1. Our first objective is to generate Sod1$^{-/-}$ MEFs that do not originate from Sod1$^{-/-}$ mice. Indeed, the survival of these mice and the characteristics of their MEFs could result from having gone through development without the Sod1 gene. For this we will use the Cre-LoxP system. We previously obtained a mouse strain carrying a floxed Sod1 gene which can be knocked out by introducing the Cre recombinase via viral infection. The validation of the presence of a Sod1 deletion was ascertained by western blotting for SOD1. The second objective is finding compounds that can rescue Sod1$^{-/-}$ MEFs from cell death or senescence or both. The effectiveness of one compound over the other will reveal Sod1's role in different signaling pathways leading to cell viability or death.

POSTER 42 – Transferrin receptors 1 and 2 are dispensable for iron supply to hepatocytes

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Most tissue cells take up iron from circulating transferrin via transferrin receptor 1 (Tfr1), which is ubiquitously expressed and is considered as the cellular iron gate. Transferrin receptor 2 (Tfr2), a Tfr1 homologue, is primarily expressed in hepatocytes and erythroid progenitor cells. It has ~25-fold lower affinity for transferrin and its main function is to control signaling to the iron hormone hepcidin in hepatocytes, and sensitivity to erythropoietin in erythroid cells. Disruption of the Tfr1-encoding Tfrc gene is linked to early embryonic lethality in mice, while inactivation of the Tfr2-encoding Tfr2 gene causes systemic iron overload (hemochromatosis) in mice and humans; this phenotype is recapitulated in hepatocyte-specific $Tfr2^{-/-}$ mice. On the other hand, hepatocyte-specific $Tfrc^{-/-}$ mice exhibit low liver iron stores and are predisposed to iron deficiency anemia. To explore whether Tfr2 plays a critical role in hepatocellular iron supply in the absence of Tfr1, we generated double hepatocyte-specific $Tfrc^{-/-}Tfr2^{-/-}$ mice by breeding floxed $Tfrc^{fl/fl}$ and $Tfr2^{fl/fl}$ animals, and subsequently crossing them with AlbCre transgenics. The resulting hepatocyte-specific $Tfrc^{-/-}Tfr2^{-/-}$ mice are viable and do not exhibit any apparent phenotypic abnormalities. Molecular characterization revealed that these animals develop hemochromatosis and essentially phenocopy single hepatocyte-specific (or full) $Tfr2^{-/-}$ mice with regard to liver iron content, serum iron biochemistry, hepcidin levels, and capacity to regulate hepcidin expression in response to dietary and pharmacological iron challenges. Considering that the Alb promoter driving the Cre recombinase is first activated in the fetal stage, our data suggest that transferrin receptors 1 and 2 are dispensable for iron supply to hepatocytes, even prenatally. Biochemical experiments are underway to determine whether hepatocytes deficient in both Tfr1 and Tfr2 can internalize transferrin-bound iron.

POSTER 43 – Evaluating the role of lipid-derived electrophiles in ferroptotic cell death using fluorescence microscopy

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Lipid-derived electrophiles (LDEs) are reactive species formed during the breakdown of lipid hydroperoxides. Despite evidence for increased LDE levels during ferroptosis, a potential role for altered LDE metabolism in ferroptotic cell death had not been evaluated. Intrigued by the role LDEs play in ferroptosis pathology, we have recently developed a method using a fluorogenic (turn-on) LDE mimic and live cell fluorescence microscopy to monitor LDE metabolism in real time. Here we describe the application of this method, where we discovered that the detoxification pathway that operates to protect against LDE-mediated damage in healthy cells becomes impaired during ferroptosis. This LDE detoxification failure amplifies cellular electrophile accumulation and increases cell death susceptibility. We further describe extensions of our work with fluorogenic LDE mimics towards exploiting cell susceptibility to LDEs and the induction of lipid peroxidation. Our work expands the chemical biology toolkit for studying ferroptosis and sheds light on the molecular mechanisms of ferroptosis downstream of lipid hydroperoxide accumulation.

POSTER 44 – The inefficacy of oral Coenzyme Q10 in treating primary CoQ deficiency and the solution to the problem

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Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone-10, is necessary for mitochondrial respiration by functioning as a mobile carrier for the transfer of electrons from respiratory complexes I, II, and several other dehydrogenases to complex III. CoQ_{10} is also implicated in superoxide generation from the electron transport chain and as a membrane antioxidant. Mutations in CoQ_{10} biosynthetic genes cause primary CoQ_{10} deficiency, a clinically heterogeneous and rare disorder that mostly manifests as a mitochondrial disorder. Furthermore, a variety of conditions have been found to be associated with secondary CoQ_{10} deficiency. Such conditions include mutations in mitochondrial DNA and the myopathy resulting from cholesterol-lowering statin drugs, as well as a variety of other conditions. CoQ_{10} is a popular health supplement and is often recommended as a supplement to mitochondrial disease patients and to treat other conditions, such as heart failure and neurodegenerative diseases. Its key role in cellular energy metabolism and its antioxidant effect are the rationales given for these uses. Our works with unique CoQ mouse deficiency models suggest that CoQ might not act as an antioxidant in vivo. Furthermore, by systematically reviewing the outcomes of CoQ_{10} treatment in primary CoQ_{10} deficiency patients, who should be the most amenable to CoQ_{10} treatment, we were led to conclude that currently available oral CoQ_{10} replacement therapy has no, or only minimal, efficacy. To remedy this situation, we have developed a new formulation for the intravenous administration of CoQ-$_{10}$. Our formulation, which uses only FDA-approved components, increases CoQ_{10} aqueous solubility 60,000 times. In contrast to oral administration, intravenous administration with this formulation leads to extreme supraphysiological concentration of CoQ_{10} in mouse plasma and tissues.

POSTER 45 – Attenuation of Oxidative Stress and Improving Kidney Function by Folate in Acute Kidney Injury

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Oxidative stress plays a significant role in the pathogenesis of kidney disease. Acute kidney injury (AKI) is defined by a rapid decline in kidney function over a short period of time. Despite adequate dietary intake, patients with AKI, chronic kidney disease, or end-stage renal disease frequently exhibit a deficiency of circulating folate/folic acid, a micronutrient that is essential for various metabolic processes and has antioxidative effects. Kidney efficiently reabsorbs folate through a transporter-mediated process in proximal tubules to prevent the urinary loss of folate. In our previous study, we found a negative correlation between plasma creatinine, a marker of kidney dysfunction, and plasma folate levels in rats with ischemia-reperfusion (IR)-induced AKI. This study investigated the effect of 5-methyltetrahydrofolate (5-MTHF), the primary form of folate in the circulation, on kidney function/injury and oxidative stress in rats with IR-induced AKI. Sprague-Dawley rats developed AKI after the kidney was clamped for 45 min followed by 24 h of reperfusion. Injection of 5-MTHF (3 μg/kg body weight) improved kidney function and decreased the expression of neutrophil gelatinase-associated lipocalin (NGAL), a marker of kidney proximal tubular injury. The restoration of glutathione and reduction of lipid peroxidation in the kidney revealed improved oxidative stress by 5-MTHF. Activation of nuclear factor erythroid 2-related factor 2 (Nrf-2) plays a crucial role in the defense against IR-induced oxidative stress in the kidney. Injection of 5-MTHF activated Nrf2 signaling and increased the expression of glutathione synthesizing enzymes as well as superoxide dismutase-1 (SOD-1) and heme-oxygenase-1 (HO-1) in the kidney. Hypoxia-reoxygenation (HR) that simulated ischemia-reperfusion caused oxidative stress, and 5-MTHF treatment (2 μg/mL) restored intracellular glutathione levels and improved NGAL expression in tubular cells injured by HR. The beneficial effects of 5-MTHF against oxidative stress were abolished when Nrf2 signaling was inhibited through Nrf2 siRNA transfection. These findings suggest that the protective effect of 5-MTHF against oxidative stress in the kidney is mediated, in part, by the activation of Nrf2 signaling. Since patients with kidney disease often have low circulating folate levels and increased oxidative stress, folate (5-MTHF) supplementation may have therapeutic potential in improving clinical outcomes in AKI.

POSTER 46 – Nuclear-Mitochondrial DNA Mismatch Induces Tissue-Specific Gene Expression Profiles: Transcriptomic Analysis of Subcutaneous and Visceral White Adipose Tissues in Mice

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Background: Cardiometabolic diseases (CMDs) are a leading cause of death worldwide. Obesity is a major risk factor for CMD, and white adipose tissue (WAT) disproportionately contributes to CMD development; visceral (v)WAT is more pathogenic than subcutaneous (s)WAT. Associations between mtDNA signature, WAT gene expression, and CMD pathogenicity are unknown.
Methods: Mitochondrial-Nuclear-eXchange (MNX) mice (opposing nDNA/mtDNA) were used to isolate mtDNA-specific effects. Six-week-old male CMD-resistant C3H/HeN ("C3H"), CMD-prone C57BL6/J ("C57"), and their MNX counterpart mice ($C3H^{nDNA}$:$C57^{mtDNA}$ "C3HMNX" and $C57^{nDNA}$:$C3H^{mtDNA}$ "C57MNX") were fed chow or high-fat-diet (HFD) for 6 weeks. sWAT and vWAT RNA sequencing was performed and transcriptomes were compared within strain and diet. Up- or down-regulation of sWAT genes was determined relative to vWAT expression, a padj<0.05 was considered significant.
Results: Analyses revealed gene expression profiles were modified by both strain and diet. Numerous mitochondrial genes were upregulated in chow-fed C3HMNX sWAT (mt-Nd2, mt-Nd4, Cox8b, Cytb, Sdha) compared to C3H; additional mitochondrial genes (mt-Co1, mt-Nd5, mt-Nd6) were upregulated in HFD-fed C3HMNX sWAT; Axin2, a mitochondrial apoptosis mediator, was downregulated. Immune cell proliferation genes (Cd74, Ighd, Ighm) and immune response regulators (Cd79b, Klrk1) were upregulated in C57MNX sWAT. Skeletal muscle process genes (Myh1, Myh4, Tnnt3) were unexpectedly upregulated in sWAT of chow-fed C57 and in HFD-fed C57MNX sWAT.
Conclusions: C3HMNX mice exhibit increased expression of sWAT mitochondrial genes, suggesting significant tissue-specific metabolic profiles not present in C3H. C57MNX mice displayed increased expression of immune modulators versus CMD-prone C57 mice, linking immune variation and CMD susceptibility. nDNA/mtDNA mismatch results in unique transcriptomic, and likely metabolomic, landscapes within the WAT depots of each strain, and dictates the transcriptional response to HFD in mice.

POSTER 47 – Role of the transcription factor NFE2L3 in a mouse model of inflammation: link to colorectal cancer sidedness and oxidative stress

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Introduction: Colorectal cancer (CRC) is the fourth most common diagnosed cancer and the second most deadly cancer in Canada. Patients with inflammatory bowel disease (IBD) are at increased risk of developing CRC. We previously showed that knockdown of the NFE2L3 transcription factor leads to a significant decrease of human colon cancer cell growth in vitro and in vivo. The sidedness, the location of the tumor within the colon, has become an important prognostic and predictive factor in CRC patients. Furthermore, oxidative stress has been associated with CRC and IBD. KEGG pathway enrichment analysis of oxidative stress markers revealed higher NOX1 and NQO1 levels in $Nfe2l3^{-/-}$ mice and higher HO1 levels in wt mice in AOM/DSS induced CRC tumors.
Hypothesis: We hypothesize that the transcription factor NFE2L3 contributes to inflammation in specific locations of the colon, and these effects are linked to oxidative stress.
Methods: Dextran sulfate sodium (DSS) was used to induce colitis in mice. DSS 3% was added to the drinking water of wild-type (wt) and $Nfe2l3^{-/-}$ FvB mice for five consecutive days. The stool was collected at day 4 and day 6 of the experiment. The expression of Lipocalin-2 (LCN2) protein, an inflammatory mediator, was measured in the stool by ELISA. At day 6, the mice were sacrificed, and the colon was sectioned in three anatomical parts (proximal, mid, and distal). RNA extraction was performed on the colon tissues for RT-qPCR (Nfe2l3, LCN2, and oxidative stress markers) and RNA-seq analyses.
Results: The increased levels of Nfe2l3 transcripts in the distal colon were suppressed by the induction of inflammation in wt mice. LCN2 gene expression was increased in all colon sections after DSS treatment except in the distal colon of $Nfe2l3^{-/-}$ mice. LCN2 levels in the stool were increased at day 4 and 6 in wt and $Nfe2l3^{-/-}$ mice after DSS treatment compared to non-inflamed mice. This increase was less important in $Nfe2l3^{-/-}$ mice compared to wt mice.
Conclusions: Based on our data, we conclude that the NFE2L3 transcription factor functions as a driver of CRC in the context of an underlying inflammatory and oxidative stress inducing condition.

POSTER 48 – ER retention of proPCSK9 protects against cardiometabolic disease risk in in vitro and in vivo models

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The proprotein convertase subtilisin/kexin type 9 (PCSK9) plays an essential role inthe regulation of circulating atherogenic lipids by promoting the degradation of cell surfacelow-density lipoprotein receptors (LDLR), making it a popular target for lipid-lowering therapies.However, recent research suggests that PCSK9 may play an important role in several importantcellular stress pathways, including the regulation of the ER stress and the unfolded proteinresponse (UPR). Lebeau and colleagues (2021) have shown that ER retention of a naturallyoccurring loss-of-function (LOF) PCSK9-Q152H variant in its unprocessed zymogen state doesnot increase ER stress or activate the UPR. However, unexpectedly, this variant increasesprotein abundance of key ER resident protein chaperones such as the glucose-regulated proteins 78-kDa (GRP78) and 94-kDa (GRP94), in both cell culture and mouse models of liver-specific overexpression. Human carriers of this variant show, on average, a 79% reduction in circulating PCSK9 levels and significantly lower circulating total cholesterol and LDL cholesterolcompared to non-carriers and carriers of gain-of-function PCSK9 variants. Importantly,individuals harboring this LOF variant show no evidence of cardiometabolic disease and liverinjury in older (over 65) and very old adults (over 85). Since the inhibition of PCSK9 secretionand its retention within the ER in its zymogen state has demonstrated protective effects againstcardiometabolic diseases, we have generated a whole-body CRISPR knock-in mouse model ofthis variant, expressing endogenous levels of LOF PCSK9-Q152H. Our findings demonstrate that homozygote mice harbouring this LOF variant have lower circulating LDL cholesterol and total cholesterol levels compared to wild-type littermate controls. We aim to further investigate the intracellular effects of ER retention of LOF PCSK9 as well as its impact on cardiovascular disease risk (measured by serum cholesterol and lipid levels) and liver injury following a high-fat diet (HFD) challenge of heterozygote mice (WT/QH), homozygotes (QH/QH) and wild-typelittermate controls (WT/WT).

POSTER 49 – The role of oxidative stress in dietary carbohydrate and manganese-induced hepatic lipid metabolism in fish

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High-carbohydrate diets (HCD) and excessive manganese (Mn) intake disrupts mitochondrial homeostasis and causes oxidative stress, leading to lipid deposition and occurrence of non-alcoholic fatty liver disease (NAFLD), characterized by dramatic accumulation of hepatic lipid droplets (LDs). However, the potential molecular mechanisms are still largely unknown. In this study, we investigated the role of oxidative stress in the process of HCD or Mn overload-induced changes of hepatic lipid metabolism in yellow catfish, and to examine the process of underlying mechanisms during these molecular contexts.

In a first study, we found that HCD significantly increased hepatic lipid accumulation, induced oxidative stress and activated autophagy in yellow catfish. Using primary hepatocytes, we found that high glucose (HG) increased lipid accumulation and stimulated the release of non-esterified fatty acids (NEFAs) by autophagy-mediated lipophagy, and that lipophagy significantly alleviated HG-induced lipid accumulation. Oxidative stress played crucial regulatory roles in HG-induced lipophagy and lipid accumulation. Further experiments showed that HG-activated lipophagy and HG-induced changes of lipid metabolism were via enhancing carbohydrate response element-binding protein (ChREBP) DNA binding capacity at the PPARγ promoter region, which in turn induced transcriptional activation of the key genes related to lipogenesis and lipophagy.

In another study, we revealed that excessive Mn intake significantly increased hepatic lipid and Mn contents, decreased superoxide dismutase 2 (SOD2) activity, increased SOD2 acetylation levels, and induced mitochondrial dysfunction. *In vitro*, Mn induced mitochondrial-derived $O_2^{\cdot-}$ production through MTF1/sirtuin 3 (SIRT3)-mediated acetylation of SOD2 at the K55 and K70 residues. Additionally, mitochondrial ROS (mROS)-mediated oxidative stress was involved in Mn-induced accumulation of triglycerides; MTF1 knock-down alleviated the Mn-induced decrease in SIRT3. Mechanistically, Mn-induced lipid accumulation was via enhancing HSF1 nuclear translocation and DNA binding capacity to regions of the PPARγ promoter, which in turn induces transcription of lipogenic related target genes and subsequent lipogenesis. Our study uncovered a novel mechanism for Mn-induced lipid accumulation and mitochondrial oxidative stress via MTF1/sirt3-mediated SOD2 acetylation, and provided direct evidence for the function of HSF1 in regulating lipid metabolism through the transcriptional activation of PPARγ.

These findings emphasized the significance of autophagy and HSF1 as the possible targets for the treatment of oxidative stress-associated NAFLD.

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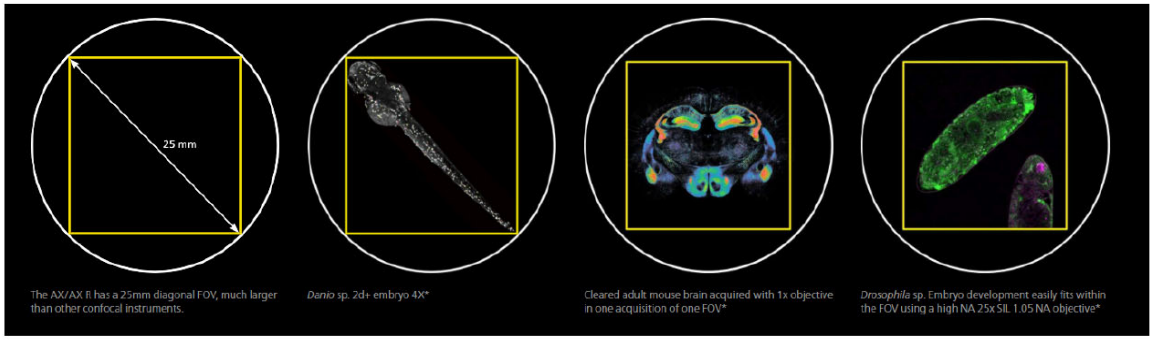

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