Speakers Abstract
All talks are scheduled at Room: Rozanski 102

SYMPOSIUM I: Metabolism

Wednesday, June 1st, 2016
10:30 AM – 12:30 PM

SA01
FUNCTION OF REDOX BUFFERING NETWORKS IN CONTROLLING MITOCHONDRIAL ROS PRODUCTION

Ryan J. Mailloux, Marisa O'Brien and Danielle Gardiner. Department of Biochemistry, Memorial University of Newfoundland.

At its core redox signaling is governed by nutrient metabolism. Electrons yielded from the combustion of carbon are utilized by mitochondria to generate reducing equivalents NADH and NADPH which trigger changes in redox buffering capacity. Although very similar in structure both molecules fulfill opposite functions. NADH is used to drive ATP formation but also gives rise to superoxide (O2●-) and hydrogen peroxide (H2O2), the dominant reactive oxygen species (ROS) produced by mitochondria. NADPH on the other hand provides reductive support, reactivating antioxidant machinery oxidized by H2O2. It has been known for some time that H2O2 can mediate redox signals, modulating various cellular functions including mitochondrial bioenergetics in response to changes in electron supply. Redox signals are sensed by “cysteine switches” protein cysteine thiols that are reversibly oxidized and reduced in response to changes in redox buffering networks. However, what remains contentious is how these signals are mediated. Indeed cysteines can undergo a series of different oxidations including sulfenylation (SUF), S-nitrosylation (SNO), and protein S-glutathionylation (PGLU). Critical examination of how redox signals can be reveals that only PGLU, thus far, can serve as a bona fide regulatory mechanism for proteins. This is evidenced by the specificity and rapid kinetics of PGLU reactions. In addition, PGLU are enzymatically mediated, unlike SUF and SNO, and are highly responsive to changes in redox buffering capacity (e.g. changes in the levels of GSH relative to GSSG which are directly affected by H2O2 levels). Mitochondria are enriched with proteins that can be reversibly modified by PGLU. Krebs cycle enzymes like α-ketoglutarate dehydrogenase (Kgdh) and respiratory chain complex NADH:ubiquinone oxidoreductase (Complex I) have been shown to be important targets. Other potential targets include pyruvate dehydrogenase (Pdh), another putative redox sensor in mitochondria. Here, I will discuss emerging evidence that PGLU is vital for controlling mitochondrial ROS formation at the levels of Kgdh and Pdh. The ability of PGLU to feedback on sites of production and limit ROS production will be emphasized. Finally, I will also present evidence illustrating that redox sensing also enhances ROS production when buffering capacity is sufficiently reducing.

SA02

MECHANISMS AND IMPLICATIONS OF MITOCHONDRIAL DYSFUNCTION IN THE SKELETAL MUSCLE AGING PROCESS

Gilles Gouspillou, Departmenté de Sciences de l'activité physique, UQAM.

Skeletal muscle aging is characterized by a progressive loss of muscle mass and strength, a process termed sarcopenia. One of the leading hypotheses to explain sarcopenia is based on the mitochondrial theory of aging, which postulates that accumulation of mitochondrial dysfunctions with aging play a causal role in muscle atrophy. The generally accepted view of this theory is that, due to the reactive oxygen species (ROS) production inherent in respiratory chain activity, aging is accompanied by the accumulation of oxidative damage to mitochondrial biomolecules, including several components of the oxidative phosphorylation machinery and mitochondrial DNA. This damage is thought to induce (i) the exacerbation of mitochondrial ROS production, (ii) an increase in mitochondrial-mediated apoptosis and (iii) an impaired capacity of mitochondria to adequately match the cellular ATP demand. However, this hypothesis remains controversial in the field. In the first part of my talk, I will present our recent data obtained in humans on aging-related accumulation of mitochondrial dysfunction as well as its implication for sarcopenia. I will then comment on the impact of muscle aging on understudied aspects of mitochondrial biology, such as mitochondrial dynamics, morphology, and mitophagy, and discuss their potential involvement in sarcopenia. Finally, I will be presenting our preliminary data on the impact of the overexpression of Parkin, a central regulator of mitophagy, on the skeletal muscle aging process.

SA03

THE ROLES OF THE STRESS ADAPTOR PROTEIN P66SHC DURING EARLY EMBRYO DEVELOPMENT

Dean H. Betts and Nicole A. Edwards, Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, The University of Western Ontario.

Mammalian embryos produced in vitro undergo high frequencies of developmental failure due, in part, to oxidative stress. The p66Shc adaptor protein controls oxidative stress response in somatic cells by regulating intracellular ROS levels through multiple pathways, including mitochondrial ROS generation and the repression of antioxidant gene expression. We have demonstrated a strong relationship with elevated p66Shc, reduced antioxidant levels and greater intracellular ROS generation associated with increased permanent embryo arrest and apoptotic frequencies of embryos cultured in atmospheric oxygen tensions or after oxidant treatment. RNA interference-mediated knockdown of p66Shc in bovine oocytes and zygotes revealed that p66Shc knockdown embryos are oxidative stress resistant displaying increased MnSOD levels, reduced intracellular ROS and DNA damage promoting successful blastocyst development. Murine blastocysts cultured under atmospheric oxygen levels have significantly increased p66Shc transcript and protein abundances compared to their in vivo counterparts. However, phosphorylated serine 36 (S36) p66Shc to total p66Shc ratio decreased in cultured mouse embryos, supporting a shift in the mitochondrial fraction of p66Shc. We have localized total p66Shc to the cell periphery of the blastocyst trophectoderm while phosphorylated S36 p66Shc displayed nuclear and cytoplasmic immunoreactivity, suggesting distinct compartmentalization of phosphorylated S36 p66Shc and the remaining p66Shc fractions. Interestingly, cultured murine blastocysts developed exhibited significantly decreased cellular ATP levels and increased superoxide production than in vivo-derived embryos. Together, these results demonstrate that p66Shc controls an

evolutionarily conserved oxidative stress response in early mammalian embryos and may differentially regulate the redox state of distinctive embryonic cell lineages. We are currently producing a conditional p66Shc knockout mouse line and utilizing the CRISPR/Cas9 genome editing system to further study the unique roles of p66Shc during early embryo development and in embryo-derived stem cell populations

SA04

SALICYLATE-BASED DRUGS: THE MECHANISM OF ACTION REVISITED

Brennan Smith, Rebecca Ford, Emily A. Day, Alex Green, Eric Desjardins, Mark Tarnopolsky, Gregory Steinberg. Department of Medicine, McMaster University.

Salsalate is a prodrug of salicylate that improves type 2 diabetes (T2D) and reduces non-alcoholic fatty liver disease (NAFLD), however the mechanism mediating these effects is unclear. Salicylate directly activates AMP-activated protein kinase (AMPK) via the β1 subunit but whether salsalate requires AMPK β1 to improve T2D and NAFLD has not been examined. Therefore, wild-type (WT) and AMPK β1 null mice (AMPK β1KO) were treated with a salsalate dose resulting in clinically relevant serum salicylate concentrations (~1 mM). Salsalate treatment increased oxygen consumption, lowered fasting glucose, improved glucose and insulin tolerance and led to an ~55% reduction in liver lipid content; effects observed in both WT and AMPK β1KO mice. Salsalate treated also lowered lipid peroxidation and increased liver mitochondrial P/O ratios. To explain these AMPK-independent effects, we show that salicylate increases oligomycin-inhibited respiration (state 4o) in permeabilized hepatocytes at subclinical concentrations (0.25 mM). This uncoupling effect is tightly correlated with the suppression of de novo lipogenesis. Salicylate is also able to stimulate brown adipose tissue respiration independent of UCP1. We propose that the primary mechanism by which salsalate improves glucose homeostasis and NAFLD is via salicylate-driven mitochondrial uncoupling to increase energy expenditure.

SA05

MECHANISMS LINKING MITOCHONDRIAL HYDROGEN PEROXIDE PRODUCTION TO SKELETAL MUSCLE INSULIN RESISTANCE IN VIVO

Daniel Lark, Department of Molecular Physiology & Biophysics, Vanderbilt University.

Increased access to foods high in saturated fat combined with decreased physical activity has increased the prevalence of metabolic syndrome worldwide. Insulin resistance (IR) in skeletal muscle (SkM) is central to the etiology of metabolic syndrome because SkM is the primary site of insulin-stimulated glucose disposal. Mitochondria are known to be critical for maintaining glucose homeostasis, but the mechanism(s) involved are incompletely understood. Consuming a high fat diet leads to increased mitochondrial oxidant production and ectopic accumulation of fat that causally contributes to IR. We have demonstrated that the membrane permeable, non-radical oxidant hydrogen peroxide (H2O2) is critical for the development of IR in rodents. These studies have also led to the discovery that mitochondrial H2O2 produced by high fat-fed mice causes SkM inflammation that leads to expansion of the extracellular matrix (ECM). Integrin receptors located on the sarcolemma respond to ECM expansion and promote IR through integrin-linked kinase (ILK). Here, I will present evidence indicating that ILK is necessary for: 1) the development of insulin resistance, 2) ectopic lipid accumulation and 3) increased fatty acid oxidation. Cumulatively, these findings implicate trans-sarcolemmal communication between the ECM and mitochondria via ILK that contributes to SkM IR. Moreover, these data suggest that high fat feeding activates

a feed-forward circuit in which mitochondrial H2O2 production accelerates fat uptake and oxidation via ILK that may both initiate and worsen IR in vivo.

SA06

HUMAN MESENCHYMAL STEM CELL ISOLATION AND CULTURE; ARE HYPOXIA MIMETIC AGENTS' SUITABLE ALTERNATIVES TO ENGINEERED OXYGEN CONTROL MEASURES?

Nicholas Forsyth, and Muhammad Ahmed. Institute of Science and Technology in Medicine, Keele University.

Human bone marrow-derived mesenchymal stem cells (hMSCs) are in current use in over 450 clinical trials globally. These trials span multiple disease states and injury situations ranging from osteoarthritic lesions of the knee to neurological indications including ischemic stroke. hMSC are canonically described as having a multipotent (osteoblast, chondrocyte, adipocyte) differentiation potential, a guideline immunophenotye (positive: CD105, CD73, CD90; and negative: CD45, CD34, CD14, CD19 and HLA-DR), and as being a plastic adherent cell isolated from the bone marrow. A key feature of bone marrow is the sinusoidal organisation of the blood vessels and the consequent low physiological oxygen levels the tissue encounters. It is now well established that defined reduced oxygen culture conditions enhance isolation and expansion of hMSC from bone marrow, a key requirement for hMSC-based cell therapies. Engineered oxygen control measures provide controlled reduced oxygen culture environments to mimic a component of the marrow niche. Hypoxia mimetic agents (HMAs) have been proposed as a cheaper alternative to engineered control measures. We have therefore evaluated the role of a panel of well-established HMAs (CoCl2, DFO, DMOG) vs. 2% O2 in hMSC isolation and culture examining frequency of colony forming unit fibroblastic (CFU-F), growth curves, nitroreductase activity (NA), oxidative stress (OS), apoptosis vs. death/necrosis, and mitochondrial copy number. hMSC CFU-F recovery from primary bone marrow was significantly inhibited in all conditions tested (21% O2, 21% O2 + HMA) vs. 2% O2 (p<0.05). No significant gains were noted for 21% O2 vs. 21% + HMA. As anticipated 2% O2 culture resulted in significant increases in both OS and NA and while CoCl2 did not elevate OS levels in 21%O2 DFO and DMOG did to levels comparable to 2% O2. CoCl2 and DFO both failed to elevate NA levels in 21% O2 while DMOG stimulated a 20% increase in NA activity above 2% O2 levels. Apoptosis was significantly elevated in 21% O2 with both CoCl2 and DFO with moderate increases observed with DMOG vs. both oxygen levels. Finally, significant reductions of mitochondrial genome copy number were noted in 2% O2 vs. 21% O2 (p<0.001) and increases in 21% O2 with all HMAs tested (p<1X10-4) accompanied by compensatory alterations in mitochondrial activity.

In summary, HMAs do not provide an accurate replication of an engineered oxygen control measures in hMSC culture and expansion. This is reflected in biological alterations which impact on cell yield, behaviour, and biology.

SYMPOSIUM II:
Graduate Student Symposium A

Wednesday, June 1st, 2016
2:00 PM – 3:20 PM

SA07
CYTOCHROME C PEROXIDASE (CCP1), A PROTEIN AT THE CROSSROADS OF THE STRESS RESPONSE AND HEME TRAFFICKING IN YEAST
Ann English[1,2,3], Meena Kathiresan[1], [1]Concordia University, [2]CBAMS, and [3]PROTEO.

Our group has demonstrated in several studies that cytochrome c peroxidase (Ccp1) is a novel mitochondrial H2O2 sensor and heme donor in yeast cells. To further elucidate its roles in cellular redox regulation and in heme trafficking, we adopted a targeted proteomics approach to screen for Ccp1 interactors. Specifically, using glutathione-S-transferase (GST) pulldown assays, combined with 1D gel electrophoresis and high-resolution mass spectrometry, we probed for interactors of apo- and holoCcp1 in extracts from 1 d fermenting and 7 d stationary-phase respiring yeast. We identified Ccp1's peroxidase co-substrate cytochrome c (Cyc1) plus 28 novel interactors including mitochondrial Mn superoxide dismutase 2 (Sod2) and cytosolic CuZn superoxide dismutase (Sod1), the mitochondrial transporter Pet9 (a putative heme transporter), the three yeast isoforms of glyceraldehyde-3-phosphate dehydrogenase (Tdh3/2/1), heat shock proteins including Hsp90 and Hsp70, and the main peroxiredoxin in yeast (Tsa1) plus its co-substrate, thioreoxin (Trx1). Identification of these new interactors suggest a key role for Ccp1 in coordinating the stress response and heme trafficking in yeast mitochondria. Thus, our proteomics results open up several new avenues of investigation.

GS01
EARLY ONSET OF MUSCLE-SPECIFIC ALTERATIONS IN MITOCHONDRIAL BIOENERGETICS IN THE D2.B10-DMDMDX/2J MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY.
Meghan C. Hughes, Sofhia V Ramos, Patrick C Turnbull and Christopher GR Perry, Muscle Health Research Center, School of Kinesiology and Health Science, York University, Toronto, ON, Canada

Duchenne Muscular Dystrophy (DMD) is a progressive muscle wasting disease resulting from mutations in the X-linked gene dystrophin. The loss of dystrophin in the dystroglycan complex causes severe muscle pathology yet the specific signaling mechanisms leading to this muscle wasting remain unclear. The mitochondria has increasingly been considered a key contributor to the pathology seen in DMD yet the characterization of specific changes occurring within the mitochondria have been limited. We hypothesized that young D2.B10-DMDmdx/2J would exhibit elevated levels of mitochondrial H2O2 emission and concurrent decreases in mitochondrial respiration. At 4 weeks of age, male D2.B10-DMDmdx/2J and DBA.2J healthy controls were sacrificed and the left ventricle, diaphragm and quadriceps muscles were removed to allow for the preparation of permeabilized muscle fibre bundles. Mitochondrial H2O2 emission was detected using Amplex UltraRed in a high resolution spectrofluorometer. In the left ventricle, complex I-derived mitochondrial H2O2 (5mM Pyruvate/2mM Malate) was 57% higher in D2.B10-DMDmdx/2J relative to healthy controls (306.2+/- 33.3 vs 194.7 +/- 26.3 pmol/sec/mg

dry wt). However, there were no differences in complex I-derived H2O2 in the diaphragm or quadriceps relative to control. Oroboros Oxygraph-2k high resolution respirometers were used to study mitochondrial respiration. Pyruvate and malate (5mM/2mM) were again used as complex I substrates followed by the addition of physiological (25µM) and supra-physiological (5mM) concentrations of ADP to stimulate state III respiration. No differences in respiration were observed in the left ventricle or diaphragm. However, quadriceps muscle from the DMD mice showed significantly lower rates of respiration at both 25µM (6.6 +/- 1.4 vs 16.5 +/- 3.6 pmol/sec/mg dry wt) and 5mM ADP (18.7 +/- 2.7 vs 53.9 +/- 15.9 pmol/sec/mg dry wt). These findings at an early age highlight the need for further characterization of the alterations in mitochondrial function associated with the progression of DMD.

GS02

EPITHELIAL DUOX2 REGULATES A SPECIFIC SUBSET OF ANTIVIRAL AND PROINFLAMMATORY CYTOKINES DURING VIRUS INFECTION

Natalia Zamorano[1,2], Espérance Mukawera, Alexa Robitaille, Karin Fink, Nathalie Grandvaux and Espérance Mukawera[1]. [1]CRCHUM; [2]Université de Montréal.

Purpose: Airway Epithelial Cells (ECs) autonomous antiviral response relies on the secretion of mucus, peptides and cytokines. The cytokine response is a major determinant of the infection outcome and orchestrates the development of an appropriate adaptive immune response. We previously found that DUOX2 expression and activity is induced at late time of infection by Sendai virus and to a lesser extent Respiratory Syncytial Virus. Our goal is to further define the role of DUOX2 in the cytokine profile produced by ECs during virus infections. Experimental design: A549 cells and primary normal human bronchial ECs (NHBE) were transfected with control- or DUOX2 specific-siRNA and infected with Sendai virus. The capacity of the cells to mount an antiviral state was determined by quantification of newly produced infectious virions. Profiling of antiviral and proinflammatory cytokines was performed by multiplex Elisa/Luminex-based assays. Results: DUOX2 activity was found to be essential for the establishment of an antiviral response. Profiling of cytokine expression revealed that DUOX2 activity is required for the sustained production of type I and III antiviral interferons. Additionally, DUOX2 positively or negatively modulates the levels of a restricted number of proinflammatory cytokines amongst a panel of 47. Conclusion: Our results unveil a role of DUOX2 in the regulation of a define subset of antiviral and proinflammatory cytokines produced by ECs upon virus infection. This supports a role of epithelial DUOX2 in the restriction of virus replication and in the recruitment and activation of immune cells at the site of infection. Project funded by CIHR

GS03

REACTIVE CARBONYL SPECIES AND THE HEAT STRESS RESPONSE DURING PLANT REPRODUCTION

Vanessa Lundsgaard-Nielsenz, Tammy L. Sage, and Dinesh Christendat. Department of Ecology and Evolutionary Biology, University of Toronto.

The accumulation of lipid peroxide-derived saturated and α,β-unsaturated aldehydes significantly damage plant cells during high temperature (HT) stress. The toxicity of these reactive carbonyl species (RCS) are the primary cause of reduced seed set at 33°C in a HT-intolerant accession of Arabidopsis. We have identified a gene, HTT

(High Temperature Tolerance) in Arabidopsis that encodes a plastid-targeted protein functioning in the detoxification of RCS. In the HT tolerant Arabidopsis Ler, HTT is expressed in the pollen, pollen remains viable, and plants produce seed at 33°C. In contrast, Cvi, which lacks viable pollen and seed set at HT, has significantly reduced levels of HTT expression. Seed set is recovered at 33 °C following complementation of Cvi with Ler HTT. The loss of pollen viability in Cvi and htt1-1 at 33°C is associated with abnormal plastid and lipid accumulation. We assessed protein carbonylation and used mass spectrometry to identify carbonylated proteins to test the hypothesis that HTT functions to maintain plastid homeostasis and pollen viability during HT stress. Pollen plastids in Arabidopsis are essential for pollen maturation due to their critical role in fatty acid and carbohydrate metabolism. Consistent with our hypothesis, carbonylated protein levels were higher in Cvi and htt1-1 than Ler at HT. A positive correlation between the accumulation of carbonylated proteins and stress-induced damage in pollen indicates a cause-effect relationship between carbonyls and the loss of pollen function. Proteomic analysis showed that Cvi and htt1-1 accumulated carbonylated proteins targeted to plastids that operate in carbohydrate metabolism, protein chaperone activity, and signaling. Other carbonylated proteins produced at HT in Cvi and htt1-1 included those involved in pollen wall synthesis, pollen tube growth, ROS detoxification, and the heat-induced signal transduction pathway. Our results provide a novel role for HTT in removing RCS to maintain pollen viability at HT.

GS04

REDUCED SUBCELLULAR LEVELS OF BREAST CANCER 1 PROTEIN (BRCA1) AND INCREASED DNA DAMAGE IN EMBRYONIC TISSUE AND FETAL BRAIN OF UNTREATED AND ETHANOL-EXPOSED BRCA1 KNOCKOUT PROGENY

Danielle Drake[1], and Dr. Peter G Wells[1,2]. [1]Department of Pharmaceutical Sciences, University of Toronto; [2]Department of Pharmaceutical Sciences & Pharmacology and Toxicology, University of Toronto[2].

DNA repair-deficient homozygous null (-/-) breast cancer 1 (***Brca1***) knockout (**KO**) mouse progeny die *in utero*, while heterozygous (+/-)littermates are believed to develop normally.Enhanced DNA oxidation, embryopathies and/or postnatal neurodevelopmental deficits have been observed in +/- *Brca1* conditional KO (**cKO**) embryos or fetuses respectively exposed in culture or*in vivo* to ethanol (**EtOH**), which enhances the formation of reactive oxygen species (**ROS**).Herein,+/- standard KO mice were mated and maternal and fetal brains were extracted on gestational day (**GD**) 17, genotyped and assessed by western blot for BRCA1 protein levels.BRCA1protein wasabout 2-fold higher in fetal vs. adult brains ($p<0.001$),and BRCA1 in fetal brain was 30-40% lower in +/- progeny vs. +/+ littermates ($p<0.05$), with differential cytosolic and nuclear deficiencies. Pregnant +/- *Brca1* KO dams were treated on GD 12 or 17 withEtOH (4 g/kg i.p.) or saline vehicle. Embryos and fetal brains were extracted 6 hr post treatment, genotyped and assessed by ELISA for the oxidative DNA lesion 8-hydroxy-2'-deoxyguanosine (**8-OHdG**), and by western blot for γH2AX formation, indicating DNA double strand breaks (**DSBs**). *Brca1*genotype effects were observed in embryos and fetal brains with and without EtOH exposure. Among progeny exposed only to saline, γH2AX was increased in +/- *Brca1* embryos and fetal brains compared to +/+ littermates ($p<0.05$), suggesting a pathogenic potential for physiological ROS levels, and that BRCA1 protects against ROS-initiated DNA damage, which may be relevant to adverse developmental outcomes like autism spectrum disorders (**ASD**). Similarly, EtOH-exposed +/- *Brca1* KOs exhibited enhanced DNA oxidation and DSBs compared to EtOH-exposed +/+ littermates, as well as to saline-exposed +/- progeny ($p<0.05$). Among EtOH-exposed progeny, the increased γH2AX and 8-OHdG levels in +/- *Brca1* embryos and fetal brains suggest

that oxidatively damaged DNA plays a pathogenic role in the mechanism of fetal alcohol spectrum disorders (**FASD**), and that BRCA1 is protective. These results indicate a broader biological role for BRCA1 (beyond cancer) in protecting the developing embryo and fetus from both physiological ROS levels and EtOH-initiated oxidative stress, respectively potentially relevant to ASD and FASD. (Support: CIHR)
Funding: Canadian Institutes of Health Research

SYMPOSIUM III: DNA Repair

Wednesday, June 1st, 2016
3:50 PM – 4:10 PM

SA08
FINALLY, A NON-CARDIOTOXIC DOXORUBICIN ANALOG
Brian B. Hasinoff[1], Xing Wu, Daywin Patel, Ragu Kanagasabai[2], Soumendrakrishna Karmahapatra[2], and Jack C. Yalowich[2]. [1]University of Manitoba; [2]College of Pharmacy, Ohio State University.

Pixantrone is a new non-cardiotoxic aza-anthracenedione anticancer drug structurally related to anthracyclines and anthracenediones such as doxorubicin and mitoxantrone. Pixantrone is approved in the European Union for the treatment of relapsed or refractory aggressive B-cell non-Hodgkin lymphoma. This study was undertaken to investigate both the mechanism(s) of its anticancer activity and its relative lack of cardiotoxicity. Pixantrone targeted DNA topoisomerase IIα as evidenced by its ability to inhibit kDNA decatenation; to produce linear double-strand DNA in a pBR322 DNA cleavage assay; to produce DNA double strand breaks in a cellular γH2AX assay; to form covalent topoisomerase II-DNA complexes in a cellular immunodetection of complex of enzyme-to-DNA (ICE) assay; and to display cross-resistance in etoposide-resistant K562 cells. Pixantrone produced semiquinone free radicals in an enzymatic reducing system, though not in a cellular system; likely due to low cellular uptake. Pixantrone was 10-12-fold less damaging to neonatal rat myocytes than doxorubicin or mitoxantrone, as measured by lactate dehydrogenase release. Three factors potentially contribute to the reduced cardiotoxicity of pixantrone. First, its lack of binding to iron(III) makes it unable to induce iron-based oxidative stress. Second, its low cellular uptake may limit its ability to produce semiquinone free radicals and redox cycle. Finally, since the beta isoform of topoisomerase II predominates in post-mitotic cardiomyocytes, and pixantrone is demonstrated here to be selective for topoisomerase IIα in stabilizing enzyme-DNA covalent complexes, the attenuated cardiotoxicity of this agent may also be due to its selectivity for targeting topoisomerase IIα over topoisomerase IIβ.

SA09
OXOGUANINE GLYCOSYLASE 1 (OGG1) AND METHYLMERCURY-INDUCED DNA DAMAGE? GIMME A BREAK!
John Peter McPherson[1], Stephanie L. Ondovcik[2], Laura Tamblyn[1], Peter G. Wells[2]. [1]Pharmacology and Toxicology, University of Toronto; [2]Pharmaceutical Sciences, University of Toronto.

The environmental contaminant methylmercury (MeHg) is a potent neurotoxin and teratogen, however the mechanistic basis of its toxicity remains unclear. MeHg exposure can generate DNA damage but the relationship of DNA damage to cellular toxicity is unknown. MeHg can also enhance oxidative stress, creating oxidative damage in DNA bases that is primarily repaired by oxoguanine glycosylase 1 (OGG1). We queried whether loss of the cellular capacity to repair oxidative base damage in DNA would sensitize cells to MeHg toxicity. Accordingly, we exposed wild-type and OGG1 null (Ogg1-/-) murine embryonic fibroblasts to environmentally relevant concentrations of MeHg and compared sensitivity via clonogenic assay, induction of apoptosis, capacity

for cell cycle arrest, generation of DNA double-strand breaks (DSBs) and propensity for formation of chromosomal aberrations. Ogg1-/- cells exhibited greater sensitivity to MeHg than wild-type controls as measured by clonogenic assay, and showed a greater propensity for MeHg-induced apoptosis. Both wild-type and Ogg1-/- cells underwent cell cycle arrest when exposed to micromolar concentrations of MeHg. Although OGG1 serves to rectify oxidative damage to DNA bases, surprisingly the extent of DSBs was exacerbated in Ogg1-/- cells compared to wild-type controls. Pre-treatment with the antioxidative enzyme catalase reduced levels of DSBs in both wild-type and Ogg1-/- cells, but failed to rescue MeHg-initiated apoptosis. Compared to exponentially growing cells, cells seeded at higher densities exhibited compromised proliferation, which attenuated MeHg-mediated cell cycle arrest and induction of DSBs in DNA. Taken together, our findings: (i) implicate reactive oxygen species-mediated DNA damage in the mechanism of MeHg toxicity, (ii) demonstrate for the first time that impaired DNA repair capacity enhances cellular sensitivity to MeHg, suggesting that the genotoxic properties of MeHg may underlie its neurotoxic and teratogenic effects, (iii) show that Ogg1 safeguards against MeHg-induced double-strand breaks and chromosomal instability, and (iv) indicate proliferative capacity governs cellular susceptibility to MeHg toxicity. Our findings support a novel role for OGG1 in protection against MeHg-induced DNA lesions that trigger replication-associated DSBs in DNA and suggest that proliferative capacity may determine MeHg toxicity in vivo and in utero. (Support: CIHR)

SA10

EXERCISE-INDUCED MITOCHONDRIAL P53 REPAIRS MTDNA MUTATIONS IN MUTATOR MICE

Adeel Safdar[1,2], Ayesha Saleem[3], Sandeep Raha[3], Konstantin Khrapko[2], Michael De Lisio[4], Adam P. W. Johnston[4], Gianni Parise[4], and Jonathan P. Little[5]. [1]Departments of Kinesiology, Pediatrics, Medicine, McMaster University; [2]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA; [3]Department of Pediatrics, McMaster University; [4]Department of Kinesiology, McMaster University; [5]School of Health and Exercise Sciences, University of British Columbia Okanagan.

Human genetic disorders and transgenic mouse models have shown that mitochondrial DNA (mtDNA) mutations, systemic oxidative stress, and telomere dysfunction instigate the aging process. Epidemiologically, exercise is associated with greater life expectancy and reduced risk of chronic diseases. While the beneficial effects of exercise are well established, the molecular mechanisms instigating these observations remain unclear. Endurance exercise reduces mitochondrial DNA (mtDNA) mutation burden, alleviates multisystem pathology, and increases lifespan of the mutator mice, with proofreading deficient mitochondrial polymerase gamma (POLG1). We report evidence for a POLG1-independent mtDNA repair pathway mediated by exercise, a surprising notion as POLG1 is canonically considered to be the sole mtDNA repair enzyme. Here we show that endurance exercise promotes translocation of the tumour suppressor protein p53 to mitochondria, which results in induction of PGC-1a that culminates into induction of mitochondrial biogenesis and cellular antioxidant enzymes that mitigate systemic oxidative stress. Additionally, mitochondrial p53 facilitates mtDNA mutation repair, which together with increased cellular redox capacity mitigates systemic pathology and increased lifespan of the mutator mice. Indeed, in mutator mice with muscle-specific deletion of p53, exercise failed to prevent mtDNA mutations, induce mitochondrial biogenesis, preserve mitochondrial morphology, reverse sarcopenia, or mitigate premature mortality. Our data establish a new role for p53 in exercise-mediated maintenance of the mtDNA genome, and presents mitochondrially-targeted p53 as a novel therapeutic modality for diseases of mitochondrial etiology.
Funding: This work is supported by CIHR Banting Fellowship and CIHR Operational Grant.

SA11

NEUROTOXICITY INITIATED BY NUCLEOTIDE EXCISION REPAIR DNA LESIONS IS RESCUED BY 2'-DEOXYNUCLEOSIDES

Rebecca Laposa, Nishani Rajakulendran, and Laura Tamblyn. Department of Pharmacology and Toxicology, University of Toronto.

The accumulation of oxidative damage, particularly bulky oxidative DNA damage that is a substrate for nucleotide excision repair (NER), has been hypothesized to be a molecular cause of brain aging, and a molecular cause of neurodegeneration in DNA repair-deficient individuals. We interrogated the neuronal DNA stress response to DNA lesions that are NER substrates. In neurons, DNA damage (breaks) can initiate inappropriate cell cycle re-entry. We hypothesized that DNA lesions which are substrates for nucleotide excision repair would also trigger neuronal cell cycle re-entry. We irradiated neurons with UVC DNA damage (10 or 50J/m2) and then added the nucleoside analogue bromodeoxyuridine (BrdU) to monitor cell cycle re-entry. Unexpectedly, BrdU prevented UV-initiated apoptosis in neurons, and cell cycle re-entry was not detectable. Normal 2'-deoxynucleosides thymidine, guanosine or cytidine also protected against neuronal apoptosis, suggesting that an increase in the repair synthesis step of nucleotide excision repair (NER) protects against UV-initiated neuronal apoptosis. BrdU did not change the removal of UV-induced DNA lesions ((6-4) photoproducts and cyclobutane pyrimidine dimers) in neurons, a step in NER that occurs prior to repair synthesis. We hypothesized that Xpa-/- neurons, which are deficient in NER at a step prior to repair synthesis, would be unaffected by BrdU treatment following UV-irradiation. Xpa-/- neurons did not undergo apoptosis at 18 or 24 hours after UV-irradiation, a time at which apoptosis was readily apparent in wild type neurons. UV-initiated apoptosis was delayed in Xpa-/- neurons; these died at 48-72 hours post-irradiation. Taken together, these data indicate that 2'-deoxynucleoside supplementation after UV irradiation prevents neuronal apoptosis while Xpa controls the kinetics of the neuronal apoptotic response.

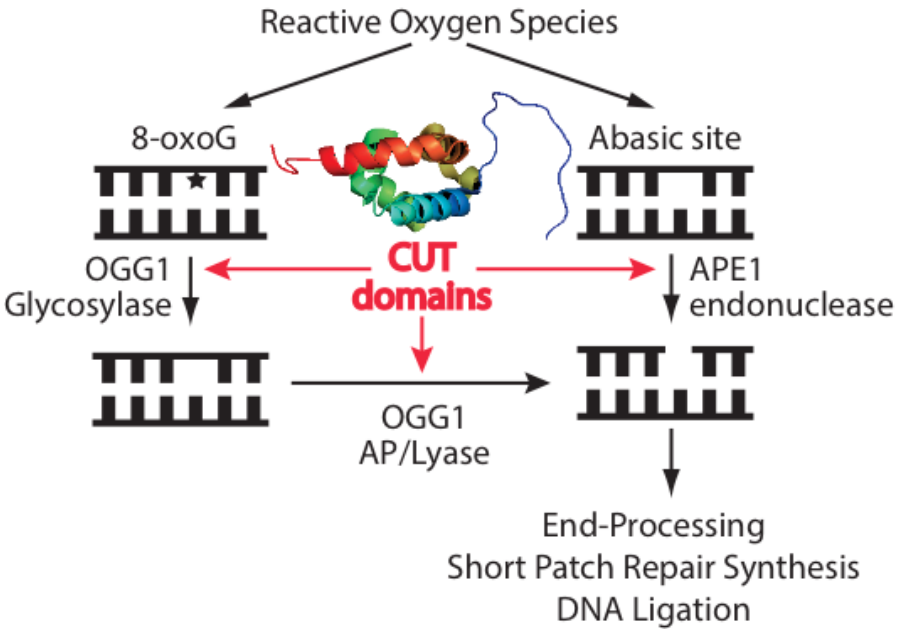
SA12

CUX1 FUNCTIONS AS AN ACCESSORY FACTOR IN THE REPAIR OF OXIDATIVE DNA DAMAGE

Alain Nepveu, Zubaidah M. Ramdzan[1], Caroline M. Donovan, Simran Kaur, Jordan B. Pinder[2], Graham Dellaire[2], and Angelo Iulianella[3]. [1]Goodman Cancer Research Centre, McGill University; [2]Department of Pathology, Dalhousie University; [3]Departments of Medical Neurosciences, Dalhousie University.

Deficient repair of oxidative DNA damage has been implicated as a causal factor in cancer development, neurodegenerative diseases, cellular senescence and aging. In contrast, more efficient repair mechanisms increase tumor resistance to radiotherapy, a treatment that aims to kill cancer cells by causing an excess of oxidative DNA damage. DNA lesions produced by reactive oxygen species include oxidized bases, apurinic/apyrimidinic (AP) sites and single-strand breaks (SSBs), which are all repaired by the base excision repair (BER) pathway. BER is initiated by one of many DNA glycosylases that recognize and remove specific types of altered bases to produce an apurinic/apyrimidinic (AP) site. The AP endonuclease 1, APE1, then incises the DNA backbone. This is followed by end-processing of the resulting single-strand break and DNA synthesis to fill the gap. CUT domains, also called Cut repeats, are present in three copies in the CUX1 protein. We previously demonstrated that CUT domains stimulate the 8-oxoguanine DNA glycosylase, OGG1. In the present study, we show that CUT domains also stimulate APE1. Accordingly, CUX1 knockdown decreases APE1 activity and increases the number of abasic sites in genomic DNA. At the cellular level, the impact of CUX1 DNA repair accessory activity is observed in multiple situations. Mouse embryo fibroblasts from Cux1-/- mice proliferate well in 3% oxygen, but rapidly

senesce when cultured in atmospheric (20%) oxygen. Increased senescence-associated β-galactosidase activity is observed in the brain of Cux1+/- heterozygous mice. CUX1 knockdown sensitizes cancer cells to radiation, whereas higher CUX1 expression confers resistance to radiation. Importantly, a recombinant protein containing only CUT domains 1 and 2 (C1C2) is sufficient for rapid recruitment to DNA damage, acceleration of DNA repair and increased resistance to treatments. Together these results firmly establish CUX1 as a key accessory factor in base excision repair and implicate the DNA repair functions of CUX1 in the response of cancer cells to radiotherapy. CUX1 gene copy number is increased in 70% of cancers and elevated CUX1 expression inversely correlates with patient survival. We propose that the role of CUX1 in DNA repair explains this unfortunate correlation.



CUT Domain DNA Repair Accessory Activity

SA13

THE EFFECT OF DELETION OF 8-OXOGUANINE GLYCOSYLASE ON AFLATOXIN B1 TUMOURIGENICITY IN MICE

Jeanne E. Mulder[1], Patricia V. Turner[2], and Thomas E. Massey. [1]Department of Biomedical and Molecular Sciences, Pharmacology; Toxicology Graduate Program, Queen's University; [2]Department of Pathobiology, University of Guelph.

Aflatoxin B1 (AFB1), a mycotoxin produced by species of Aspergillus, is a human carcinogen. AFB1 may initiate cancer by causing oxidatively damaged DNA, specifically by causing 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) lesions. Base excision repair removes these lesions, with 8-oxoguanine glycosylase (OGG1) being the rate-limiting enzyme. Female wild type, heterozygous, and ogg1 null mice (50 mice per group) were given a single dose of 50 mg/kg AFB1 or 40 µl DMSO intraperitoneally. Oxidatively damaged DNA in lungs and livers of mice was measured 2 h post-dosing, while tumourigenicity was determined up to 75 weeks post-dosing. Neither ogg1 genotype nor AFB1 treatment affected levels of oxidized guanine in lung or liver 2 h post-treatment. AFB1-treated ogg1 null mice had exacerbated weight loss and mortality relative to DMSO-treated ogg1 null mice. AFB1 treatment did not significantly increase lung or liver tumourigenesis compared with DMSO controls, regardless of ogg1 genotype, although a non-significant trend towards AFB1-treated ogg1 (-/-) mice developing tumours earlier than DMSO-treated mice was apparent. Suspect lung masses from three AFB1 treated mice were adenomas, and masses from two of the mice were osteosarcomas. No osteosarcomas were observed in DMSO-treated mice. All liver masses from AFB1-treated mice were adenomas, and one also contained a hepatocellular carcinoma. The K-ras mutation pattern typically observed with AFB1-induced mouse lung tumours was not observed, suggesting that the lung tumours were either spontaneous, or metastatic lesions from other tissues. In conclusion, deletion of ogg1 did not significantly affect AFB1-induced oxidatively damaged DNA or tumourigenicity, however, deletion of one or both alleles of ogg1 did increase susceptibility to other aspects of AFB1 toxicity (Supported by CIHR Grant No. MOP-89698).

SYMPOSIUM IV: Plants

Thursday, June 2nd, 2016
10:30 AM – 12:30 PM

SA14
STRESS AND GAMMA-AMINOBUTYRATE METABOLISM IN PLANTS: AN UPDATE
Barry J. Shelp, Department of Plant Agriculture, University of Guelph.

Gamma-aminobutyrate (GABA) is a ubiquitous non-proteinogenic amino acid that accumulates in plants in response to abiotic stresses such as hypoxia, cold, heat, drought and flooding. In this paper, I will discuss the origin of GABA from glutamate and its subsequent catabolism to succinic semialdehyde (SSA) and either succinate or gamma-hydroxybutyrate (GHB). For Arabidopsis thaliana, evidence supports the involvement of a cytosolic Ca2+/calmodulin- and pH-regulated glutamate decarboxylase (GAD), a pyruvate- and glyoxylate-regulated mitochondrial GABA transaminase (GABA-T), a redox-regulated mitochondrial succinic semialdehyde dehydrogenase (SSADH), and redox-regulated glyoxylate/succinic semialdehyde reductases (GLYR/SSAR) located in both cytosol and plastid/mitochondrion. The imposition of abiotic stress, as well as genetic or chemical disruption of GAD, GABA-T and tricarboxylic acid cycle (TCAC) reactions, indicate that that GABA metabolism and respiration are strongly linked. With hypoxia in particular, both GABA and alanine metabolism are stimulated, together with glycolysis, so that the TCAC intermediate succinate is derived from both SSA and 2-oxoglutarate. Succinate accumulates due to limited succinate dehydrogenase activity, even though SSADH activity is partially feedback inhibited, which contributes to the accumulation of GABA. The metabolic generation of GHB from SSA is probably linked to the partial inhibition of SSADH and the stimulation of GLYR activity by an increasing NADPH/NADP+ ratio. The fate of GHB remains uncertain. Recent evidence indicates that with cold treatment, glyr1/2 knockout or RNAi mutants are more sensitive to SSA than wild-type plants. These findings indicate that GABA metabolism is an adaptive mechanism for maintaining respiration and redox/energy balance during stress and recovery from stress.

SA15
THE CONTROL OF REACTIVE OXYGEN SPECIES GENERATION BY TERMINAL OXIDASES
Allison McDonald, Department of Biology, Wilfrid Laurier University.

Most scientists are convinced that the environment of the early Earth was anoxic. As such, the advent of oxygenic photosynthesis could be viewed as a catastrophe as organisms now had to effectively deal with oxygen which would have initially been toxic. While the step of water splitting yields electrons that can be used to transduce light energy into reducing power and ATP using an electron transport system, the resulting oxygen is highly reactive and readily accepts an electron to become superoxide that can damage proteins, lipids, nucleic acids, and membranes. The incorporation of cyanobacteria into a mitochondrion containing host cell via endosymbiosis has allowed plants the power of photosynthesis, but it comes at the cost of ROS generation. In a similar fashion, the electron transport system of the mitochondrion is also a site of ROS production. Levels of ROS in any compartment are the result of processes that produce ROS and the processes that convert ROS into less harmful

compounds. While much research is currently focused on the enzymes that scavenge ROS, many species use flexibility in their electron transport systems to prevent ROS generation in the first place. Plants and many other organisms contain an alternative terminal oxidase (AOX) in mitochondria and a plastoquinol terminal oxidase (PTOX) in chloroplasts. These proteins provide options for how electrons can leave the electron transport system in a controlled manner. This is hypothesized to lower the electron leak and therefore the amount of ROS generated, especially under conditions when the system is highly reduced or damaged. These ideas provide a framework for the integrative and comparative approaches used in my lab to study these two enzymes in different experimental systems and organisms under challenging environmental conditions.

SA16

LOCALIZED CONTROL OF OXIDIZED RNA

William Zerges[1], James Dhaliwal[1], Yu Zhan[1], Pauline Adjibade[2], James Uniacke[3], and Rachid Mazroui[2].
[1]Biology, Concordia University; [2]Department of Molecular Biology, Medical Biochemistry, and Pathology, LavalUniversity; [3]University of Guelph.

The oxidation of biological molecules by reactive oxygen species can render them inactive or toxic. This includes the oxidation of RNA, which appears to underlie detrimental effects of oxidative stress, aging, and certain neurodegenerative diseases. Here we investigate the management of oxidized RNA in the chloroplast of the green alga Chlamydomonas reinhardtii. Our results of immunofluorescence microscopy reveal oxidized RNA (with 8-hydroxyguanine) localized in the pyrenoid, a chloroplast microcompartment where CO2 is assimilated by the Calvin cycle enzyme Rubisco. Results of genetic analyses support a requirement for the Rubisco large subunit, but not Rubisco, in the management of oxidized RNA. An RBCL pool that could carry out such a 'moonlighting' function is revealed by results of biochemical fractionation experiments. We also show that human (HeLa) cells localize oxidized RNA to cytoplasmic foci which are distinct from stress granules, processing bodies, and mitochondria. Our results suggest that the compartmentalization of oxidized RNA management is a general phenomenon and therefore has some fundamental significance.

SA17

URIEDE METABOLISM IN ARABIDOPSIS IN RESPONSE TO ABIOTIC STRESS

Christopher Todd, Solmaz Irani, Maryam Nourimand and Jodi Souter. Department of Biology, University of Saskatchewan.

Oxidative stress and the generation of reactive oxygen species are common to multiple abiotic stresses in plants. Therefore, unsurprisingly, plants have metabolic and physiological responses that are shared in response to different stresses, such as drought, excess light, salinity, or cold. Allantoin, a ureide compound derived from purine oxidation, accumulates in a number of different plant species, including Arabidopsis , in response to abiotic stress. Using an allantoinase-negative Arabidopsis line we show that these mutants, which accumulate elevated allantoin levels, appear resistant to a wide range of abiotic stresses including water limitation, excess light, salinity, and heavy metals. Allantoinase mutants do not show the same increase in reactive oxygen species in leaf tissue as wild-type in response to abiotic stresses and exhibit increased photosynthetic efficiency. How allantoin protects plant cells is still under debate, but our results point to a possible role in activating the abiotic stress response and scavenging hydrogen peroxide and superoxide.

SA18

PLANT CELLULAR REDOX RESPONSES TO CATERPILLAR HERBIVORY: A TALE OF TWO CITIES

Jacqueline Bede, Jamuna Paudel, Alberto Prado, and Alexandre Amirizian. Department of Plant Science McGill University.

In plants, early changes to biotic stresses, such as caterpillar herbivory, include shifts in the cellular redox status. At the wound site, a rapid increase in hydrogen peroxide (H2O2) is observed. In addition, some caterpillar species, such as the noctuid beet armyworm, Spodoptera exigua, have redox-related enzymes in their labial secretions, such as glucose oxidase (GOX); this enzyme produces H2O2 via the oxidation of glucose. Cellular mitigation of elevated H2O2 levels may be mediated through the Foyer-Halliwell-Asada cycle. In Medicago truncatula plants infested by S. exigua, caterpillar labial saliva-specific increases in the oxidized-to-reduced ascorbate and glutathione are observed within the first 45 minutes post-herbivory. Ethylene signaling is necessary for these changes in redox status. On the other hand, in Arabidopsis thaliana, early changes in redox state are not seen. Instead, caterpillar labial saliva was thought to help maintain a general reductive cellular state. Though these two contrary results might be explained by plant-specific strategies, alternative theories that are currently being investigated will be presented.

SA19

ALTERNATIVE OXIDASE RESPIRATION PRESERVES BOTH MITOCHONDRIAL AND CHLOROPLAST FUNCTION DURING DROUGHT STRESS

Greg Vanlerberghe and Keshav Dahal. Department of Biological Sciences, University of Toronto Scarborough.

The plant mitochondrial electron transport chain (ETC) terminates at either cytochrome (cyt) oxidase or alternative oxidase (AOX). In Nicotiana tabacum leaves, mitochondrial respiration in the light (RL) declined with increasing drought severity but then increased under intense drought, despite a steep decline in cyt oxidase activity. This increased RL was absent in transgenic AOX knockdowns, while AOX overexpressors showed enhanced RL relative to wild-type (WT). Under intense drought, cyt oxidase activity was higher in overexpressors and lower in knockdowns, compared to WT. Chloroplast metabolism also differed across the plant lines since RL strongly influenced the reduction state of the photosynthetic ETC. Under intense drought, photosystem II efficiency was compromised in knockdowns but preserved in overexpressors compared to WT. Differences in mitochondrial and chloroplast function correlated with differences in protein carbonylation within each organelle. Hence, AOX acts to reduce oxidative damage and preserve both mitochondrial and chloroplast function during intense drought.

SYMPOSIUM V:
Graduate Student Symposium B

Thursday, June 2nd, 2016
2:00 PM – 3:05 PM

SA20
IS OXIDATIVE STRESS METABOLISM ASSOCIATED WITH PHYSIOLOGICAL INJURIES IN POME FRUIT?
Gale Bozzo, Department of Plant Agriculture, University of Guelph.

Pome fruit, such as apple and pear, undergo ethylene-mediated ripening leading to senescence. These physiological processes can be limited by controlled atmosphere storage, which is comprised of chilling in combination with low oxygen (hypoxia) and elevated carbon dioxide, and application of the ethylene antagonist, 1-methylcyclopropene. A drawback of these practices, including the use of sub-optimal temperatures and atmospheric partial pressures, is their capacity to promote the development of physiological disorders in apple and pear fruit, including flesh browning and cavities. The biochemical basis for these physiological disorders is little understood. Oxidative stress metabolites tend to accumulate (e.g., gamma-aminobutyrate) or rapidly decline (e.g., ascorbate and glutathione) in vegetative tissues, such as Arabidopsis thaliana, in response to chilling, hypoxia and/or elevated carbon dioxide. These metabolic shifts are associated with altered energy status (e.g., NADPH/NADP+). Moreover, there is little information on the manner in which the aforementioned oxidative stress metabolites are co-coordinately affected by abiotic stress, including controlled atmosphere storage-related stresses. Our laboratory is testing the hypothesis that a malfunction in oxidative stress metabolism is associated with development of physiological injuries in pome fruit during controlled atmosphere storage. The talk will focus on recent evidence that gamma-aminobutyrate accumulation and depletion of total glutathione were associated with controlled atmosphere-related injury (browning and lens-shaped cavities of the flesh) in ‘Honeycrisp’ apples during prolonged elevated carbon dioxide storage. In another experiment, a decline in total glutathione concentrations was associated with senescence-related injuries (e.g., internal breakdown) in European pear fruit; by contrast, a decline in total ascorbate concentration preceded the development of flesh cavities in fruit of the pear cultivar ‘AC Harrow Crisp’ during elevated carbon dioxide storage. This information will improve our understanding of the relationship between oxidative stress metabolism and storage-related injuries in pome fruit.

GS05

S-GLUTATHIONYLATION OF TWO CYS RESIDUES IN ARABIDOPSIS THALIANA CYTOSOLIC TRIOSEPHOSPHATE ISOMERASE

Sébastien Dumont[1], Natalia V. Bykova[2], Sonia Dorion, and Jean Rivoal. [1]Institut de Recherche en Biologie Végétale, Université de Montréal; [2]Morden Research and Development Centre, Agriculture and Agri-Food Canada

In plants, many biotic and abiotic stresses lead to abnormal accumulation of reactive oxygen species (ROS). ROS are also generated as normal by-products of many cellular processes; however, high levels of ROS can damage cell macromolecules such as nucleic acids, lipids and proteins. An increase in ROS concentrations results in a more oxidizing redox potential cellular environment and promotes S-glutathionylation, a post-translational modification resulting from the formation of mixed disulfide between glutathione and sensitive protein Cys residues. Our research focuses on the S-glutathionylation of Arabidopsis thaliana cytosolic triosephosphate isomerase (cTPI). cTPI is a housekeeping glycolytic enzyme that catalyses the interconversion between glyceraldehyde-3-phosphate and dihydroxyacetone. We showed that recombinant cTPI is sensitive to inhibition by hydrogen peroxide, diamide and physiological concentration of oxidized glutathione (GSSG). We used LC-MS/MS to further demonstrate that GSSG induces S-glutathionyation of two cTPI on Cys residues (Cys127 and Cys218). We used site directed mutagenesis to produce three mutants of cTPI (C127S, C218S and C127/218S) in which targeted Cys residues were modified to Ser. In recombinant proteins, mutation of Cys residues led to a significant loss of cTPI activity, modification of protein conformation and alteration of enzyme redox stability. The mutation of Cys127 caused a greater disruption in the enzyme parameters than that of Cys218. Comparison of biotinyl glutathione ethyl ester (BioGEE) binding by wild type and mutant proteins showed partial binding by single mutants and loss of binding by the double mutant. We also show that this modification is reversible with DTT. In vivo labelling of A. thaliana cell cultures with BioGEE followed by LC-MS/MS analysis allowed the identification of cTPI as well as 700 other proteins as possible targets of S-glutathionylation. Our study provide the first identification of the amino acid residues involved in A. thaliana cTPI S-glutathionylation. Knowing that low cTPI activity in transgenic roots results in a rerouting of carbon flux through the pentose phosphate pathway, we hypothesize that modification of cTPI by glutathione could be part of an oxidative stress response in plant cells.

GS06

METABOLIC ALTERATIONS IN AGING NEURONS: EVIDENCE LINKING PLASMA MEMBRANE LIPID PEROXIDATION AND MITOCHONDRIAL DYSFUNCTION

Jonathon R. Lee, Petra M. Hermann, and Willem C. Wildering. Department of Biological Sciences, University of Calgary.

The gradual failure of neurons in senescence is thought to involve the accumulation of oxidative stress to cellular components over the lifetime of an organism. Under the guise of plasma membrane lipid peroxidation, oxidative stress seems to directly contribute to declining neuron excitability of aging Lymnaea stagnalis. We have shown that phospholipase A2 (PLA2), an enzyme responsible for excising peroxidised free fatty acids from the plasma membrane, is tightly coupled to this neuronal failure and that its inhibition can restore the excitability of aged or young-oxidized neurons. The deleterious effects of age-associated oxidative stress are known to begin with an acquired imbalance in cellular reduction-oxidation (redox) control, though it remains unclear where this problem originates. Importantly, both sides of this redox balance are supported by mitochondria, and it is known that age-related oxidative damage can be propagated by mitochondrial dysfunction and increasing metabolic constraints with time. While mitochondria are historically viewed as the perpetrators in age-related oxidative stress due to their central role in redox homeostasis, it is possible that these organelles are not culprits, but rather victims of an oxidative shift originating in the plasma membrane. Using autofluorescent biomarkers of oxidative metabolism (NADH and FAD) and indicators for mitochondrial membrane potential (TMRM), we demonstrate in the model system Lymnaea stagnalis that, as with the progression of age-associated changes in neuronal excitability, alterations in mitochondrial activity also coincide with lipid peroxidation of the plasma membrane. NADH/FAD autofluorescence differs between young and old Lymnaea brains, indicating an intrinsic age-dependent difference in metabolic handling of FFA moieties. We also show that mitochondria of old neurons are more depolarized relative to those of young, suggesting mitochondrial inefficiency. By inducing plasma membrane lipid peroxidation, this aged phenotype can be expressed in young neurons, establishing a clear connection between the plasma membrane and metabolic control. Moreover, inhibition of PLA2 demonstrates that FFA abstraction plays an important role in this link. As in the case of age-related neuronal excitability decline, we provide evidence that plasma membrane lipid peroxidation and PLA2 activity may be important factors in progressive metabolic insufficiency and functional decline of normal aging neurons.

GS07

AMELIORATION OF NEURODEGENERATIVE DISEASE RELATED SYMPTOMS AND PATHOLOGY BY UBISOL – Q10

Krithika Muthukumaran[1], Alexandra Marginean, Austin Elliott, Samantha Leahy, Jessica Smith, Nicholas Guilbeault, Jerome Cohen[2], Marianna Sikorska, and Siyaram Pandey. [1]Department of Chemistry and Biochemistry, University of Windsor; [2]National Research Institute, Ottawa.

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) commonly affects people 60 years and above. Since the current treatment options only provide symptomatic relief and there is no effective way to halt the disease progression, there is a potential global socioeconomic crisis. Both AD and PD could be caused due to genetic disposition or environmental factors, however, oxidative stress has been implicated as a major contributor to the pathophysiology of the disease. Hence, an ideal way to halt the

disease progression would be to use an antioxidant which can scavenge the reactive oxygen species and protect the neurons. In vitro studies with a water soluble formulation of CoQ10 (Ubisol-Q10) reverses premature senescence in AD fibroblasts. Ubisol-Q10 was administered over a 14 month period to a transgenic mouse model of AD containing human amyloid precursor protein and a mutant presenilin – 1. Treatment prevented emotional and memory related behaviour impairments in the Y-maze which correlated with successful removal of plaques in the brain sections when subjected to immunohistochemistry, reduced circulating human amyloid beta, and astrocytes activation in comparison to the untreated group. Ubisol – Q10 has also been shown to provide neuroprotection both in vitro and in vivo in a paraquat rat model of PD. We have shown that the paraquat rat model is an ideal environmental toxin model and it mimics slow progressive neurodegeneration observed in patients with PD. Ubisol – Q10 alleviated symptoms and prevented loss of dopaminergic neurons when administered prophylactically and therapeutically to the paraquat rat model of PD. Neuroprotection was evaluated by counting the number of dopaminergic neurons in the substantia nigra region using stereology software following immunohistochemistry and comparing the number of neurons across the different experimental groups. Additionally, Ubisol-Q10 also provides neuroprotection when administered prophylactically in a genetically susceptible DJ-1 deficient transgenic mouse model exposed to MPTP. Ubisol - Q10 has shown higher bioavailability, even at doses much lower than that of the oil soluble formulation of CoQ10, and at 10 times lower than the FDA approved dose, and the neuroprotection properties hold promise in providing effective treatment to those suffering from these diseases.

SYMPOSIUM VI: Cancer

Thursday, June 2nd, 2016
3:35 PM – 5:15 PM

SA21
THE ONCODYNAMIC ROLE OF OXIDATIVE STRESS
Gurmit Singh and Robert Ungard. Department of Pathology & Molecular Medicine, McMaster University.

Increased mitochondrial ROS production by cancer cells is associated with a corresponding upregulation of cellular antioxidant defence mechanisms. The synthesis of glutathione is significantly increased by the enhanced uptake of rate-limiting cysteine through the action of the cystine/glutamate antiporter system xC-, which is frequently upregulated in cancer cells. We have found that the abundant glutamate excreted as a byproduct of cystine uptake through system xC- in cancer cells contributes to several significant pathologies including pain, depression, and disruption of bone cell signalling in metastatic tumours in the bone. We have coined the term "Oncodynamics" to define the impact of abnormal cues generated by tumours on the physiological functioning of the body. In this presentation we will discuss the role of oxidative stress specifically in the context of cancer-induced pain and cancer-induced depression. This shift in paradigm promotes examination of the biological mechanisms of the effects of cancer on patient quality of life. Furthermore, it provides a framework to study new mechanism-based targets that impact oncodynamic effects. Simply treating pain and depression as common symptoms has not been very effective. Both pain and depression are central to quality of life for cancer patients.
(This work was supported by Canadian Breast Cancer Foundation – Ontario region).

SA22
OXYGEN AS A REGULATOR OF TUMOR CELL PLASTICITY
Lynne-Marie Postovit, Department of Oncology, University of Alberta.

Tumours contain populations of cells with stem cell like properties, and it is believed that these cells are responsible for cancer progression, resistance to therapy and metastatic potential. Stem cell-like populations are regulated by their immediate surroundings (microenvironment) characterized by proteins, immune cells, and biophysical features such low oxygen tensions. A growing body of evidence suggests that cancer cells hijack normal stem cell-associated regulatory networks in order to behave like stem cells. We have discovered that an embryonic-associated protein called Nodal maintains stem cell phenotypes in cancer, and that it promotes classical hallmarks of cancer such as angiogenesis, and metastasis. We have also found that biophysical features of a growing tumour, in particular low oxygen, can turn regular cancer cells into cancer stem cells. This lecture will describe these findings and will introduce the audience to the mechanisms by which hypoxia can drive stem cell like plasticity in cancer.

SA23
MODULATION OF REACTIVE OXYGEN SPECIES PRODUCTION BY INTEGRIN-LINKED KINASE.
Lina Dagnino, Department of Physiology and Pharmacology, Western University.

Integrin-linked kinase (ILK) is a ubiquitous scaffold protein that mediates cellular responses to integrin stimulation by ECM proteins. In the epidermis, ILK is essential for development of keratinocyte polarity, hair follicle morphogenesis, and migration of epidermal stem cells to sites of injury during regeneration. Integrin stimulation by extracellular matrix (ECM) substrates can give rise to reactive oxygen species (ROS) production, necessary for cell survival. Because ILK-deficient (ILK-KO) keratinocytes exhibit impaired adhesion and spreading to the ECM, we investigated if these defects are associated with abnormalities in ROS production. Significantly, we observed an increased ROS levels in ILK-KO cells, as measured by DCFDA fluorescence, which were accompanied by a 10-fold increase in the levels of phosphorylated histone H2A.X (gamma-H2A.X), a marker of DNA double-strand breaks. Further, increased caspase-mediated apoptosis was observed in these cells, which was decreased by treatment with antioxidants. Therefore, ILK appears to be an important modulator of keratinocyte redox state, and its absence is associated with DNA damage and, potentially, genomic instability. Supported by the Canadian Institutes of Health Research.

SA24
INHIBITION OF FATTY ACID OXIDATION SELECTIVELY ELIMINATES ACUTE MYELOID LEUKEMIA CELLS AND LEUKEMIA STEM CELLS THROUGH ROS-MEDIATED APOPTOSIS
Paul Spagnuolo, School of Pharmacy, University of Waterloo.

Acute myeloid leukemia (AML) is an aggressive malignant disease characterized by poor patient outcome and suboptimal front-line chemotherapy. To identify novel anti-AML compounds, we performed a high-throughput screen of a natural products library (n=800) and identified avocatin B as a potent and novel anti-leukemia agent. Avocatin B, at concentrations as high as 20µM, had no effect on normal peripheral blood stem cell viability. In contrast, it induced death of primary AML cells with an EC50 of 1.5-5.0 µM. Selective toxicity towards a functionally defined subset of primitive leukemia cells was also demonstrated. Avocatin B (3µM) reduced the clonogenic growth of AML progenitor cells with no effect on clonogenic growth of normal hematopoietic stem cells. Further, treatment of primary AML cells with avocatin B (3µM) diminished their ability to engraft into the bone marrow of pre-conditioned, NOD/SCID mice (t18=6.5; p<0.001). Mechanistically, we demonstrated that avocatin B accumulates in mitochondria and inhibits fatty acid oxidation through a unique mechanism whereby it reduced NAD, NADPH and glutathione leading to ROS-dependent apoptosis. Together, these results highlight a novel AML-therapeutic strategy by which mitochondria are targeted to impair cellular metabolism leading directly to ROS-mediated AML and leukemia stem cell death.

SA25

INHIBITION OF AUTOPHAGY SENSITIZES CELLS TO HYDROGEN PEROXIDE-INDUCED APOPTOSIS: PROTECTIVE EFFECT OF MILD THERMOTOLERANCE ACQUIRED AT 40°C

Diana Averill-Bates and Hou Ve, and Maureen Redza-Dutordoir. Departmenté de Sciences biologiques, Université du Québec à Montréal.

Various toxic compounds produce reactive oxygen species, resulting in oxidative stress that threatens cellular homeostasis. Yet, lower doses of stress can stimulate defense systems allowing cell survival, whereas intense stress activates death pathways such as apoptosis. Furthermore, mild thermal stress (40°C, 3 h) induces thermotolerance, an adaptive survival response that renders cells less sensitive to subsequent toxic stress, by activating defense systems like heat shock proteins, antioxidants, anti-apoptotic and ER-stress factors. This study aims to understand how autophagy and apoptosis are regulated in response to different doses of H2O2, and whether mild thermotolerance can protect cells against apoptosis by stimulating autophagy. Autophagy was monitored through Beclin-1 and LC3 expression and acid compartment activity, whereas apoptosis was tracked by caspase activity and chromatin condensation. Exposure of cells to 5 to 90 µM H2O2 for shorter times (15-30 min) transiently induced autophagy, whereas longer exposure times (1-3 h) activated apoptosis. Mild thermotolerance at 40°C enhanced activation of autophagy by H2O2. Disruption of autophagy using bafilomycin A1 and 3-methyladenine sensitized cells to apoptosis induced by 25 to 100 µM H2O2, in non-thermotolerant cells and, to a lesser extent, in thermotolerant cells. Inhibition of autophagy enhanced apoptosis through the mitochondrial, death receptor and endoplasmic reticulum pathways. Therefore, autophagy is activated by lower doses of stress and protects cells against induction of apoptosis by higher doses of H2O2. This work improves understanding of mechanisms that might be involved in toxicity of various compounds and could eventually lead to protective strategies against deleterious effects of toxic compounds. Furthermore, inhibition of autophagy could sensitise cancer cells to cytotoxic inducers of cell death by apoptosis.

SYMPOSIUM VII: Neuroscience

Friday, June 3rd, 2016
9:00 AM – 12:20 AM

SA26
AEROBIC GLYCOLYSIS: FOOD FOR THOUGHT OR ACHILLES HEEL FOR ALZHEIMER'S DISEASE
Robert Cumming, Richard Harris, Asad Lone, and Robert Bartha, Department of Biology, University of Western Ontario.

We have previously shown that nerve cell lines selected for resistance to the toxic amyloid beta (A?) peptide undergo metabolic reprogramming and rely heavily on glycolysis rather than mitochondrial respiration to meet their metabolic needs. The reliance on glycolysis, even in the presence of oxygen, is known as aerobic glycolysis or the Warburg effect and is frequently employed by cancer cells as a survival strategy to reduce ROS production and sensitivity to apoptosis. Within the brain, aerobic glycolysis, and associated lactate production, plays an essential role in synapse function and memory. Yet the role of this metabolism in the cognitive decline associated with Alzheimer's disease (AD) remains poorly understood. Using in vivo proton magnetic resonance spectroscopy and immunohistochemical analysis of brain tissue, we examined the relationship between aerobic glycolysis and memory performance over a 12 month period in an APP/PS1 mouse model of AD which progressively accumulates A?. We detected an age-dependent decline in the expression of aerobic glycolysis enzymes and a concomitant decrease in lactate levels within the frontal cortex of wild-type mice. Improved memory performance in wild-type mice correlated with elevated expression of aerobic glycolysis enzymes. Surprisingly, lactate levels remained elevated with age and increased aerobic glycolysis enzyme expression correlated with poorer memory performance in APP/PS1 mice. These findings suggest that while lactate production is beneficial for memory in the healthy aging brain, it might be detrimental in an Alzheimer's disease context. We are currently exploring if activation of p66Shc, an adaptor protein implicated in metabolism, redox signalling and longevity, plays a role in regulating the age-dependent decline in cerebral aerobic glycolysis and neuronal sensitivity to A?.

SA27
SCAVENGING REACTIVE OXYGEN SPECIES INITIATES GABAA RECEPTOR-MEDIATED ELECTRICAL SUPPRESSION IN ANOXIA-TOLERANT TURTLE NEURONS
Les Buck and David Hogg. Department of Cell and Systems Biology, University of Toronto.

Anoxia induces hyper-excitability and cell death in mammalian brain but in the anoxia-tolerant western painted turtle (Chrysemys picta bellii) neuronal electrical activity is suppressed (i.e., spike arrest), adenosine triphosphate (ATP) consumption is reduced, and cell death does not occur. Electrical suppression is primarily the result of enhanced γ-aminobutyric acid (GABA) transmission; however, the underlying mechanism responsible for initiating oxygen-sensitive GABAergic spike arrest is unknown. In turtle pyramidal neurons there are three types of GABAA receptor-mediated currents: spontaneous inhibitory postsynaptic currents (sIPSCs), giant IPSCs and tonic currents. The aim of this study was to assess the effects of reactive oxygen species (ROS) scavenging on these three currents since ROS levels naturally decrease with anoxia and may

serve as a redox signal to initiate spike arrest. We found that anoxia, pharmacological ROS scavenging, or inhibition of mitochondrial ROS generation enhanced all three types of GABA currents, with tonic currents comprising ~ 50% of the total current. Application of hydrogen peroxide inhibited all three GABA currents, demonstrating a reversible redox-sensitive signaling mechanism. We conclude that anoxia-mediated decreases in mitochondrial ROS production are sufficient to initiate a redox-sensitive inhibitory GABA signaling cascade that suppresses electrical activity when oxygen is limited. This unique strategy for reducing neuronal ATP consumption during anoxia represents a natural mechanism in which to explore therapies to protect mammalian brain from low-oxygen insults.

SA28

LIPID PER OXIDATION, FREE FATTY ACIDS AND NEURONAL AGING

Willem Wildering, Adam Lognon, Jonathon Lee, and Petra Hermann. Department of Biological Sciences, University of Calgary.

The significance of free fatty acids (FFA) and fatty acid-derived signaling moieties in the regulation of neuronal functions is well established. Likewise a substantial body of evidence supports the view that lipid peroxidation and oxidative stress-induced release of FFAs or lipid peroxidation byproducts are important dimensions of both the normal biological aging and neuro-degenerative processes affecting the aging brain. While there is a broad consensus that lipid metabolism plays at best a minor role in neuronal energy homeostasis, remarkably little is known about its significance in maintaining cellular FFA homeostasis.In recent years, we have accumulated evidence that FFA release following plasmamembrane lipid-peroxidation induced recruitment of phospholipase A2 (PLA2) activity, an enzyme catalyzing excision of FFAs from the sn2 position of phospholipids, is associated with neuronal hypoexcitability and long-term memory (LTM) impairment characterizing normal biological aging in the snail Lymnaea stagnalis (Hermann et al., 2014). The many identified neurons and functionally characterized neural circuits of this model system allows us to directly link behavioral (dys)functions to their underlying molecular and cellular correlates. Our recent studies focused on characterizing the mechanisms through which PLA2 produces these neurophysiological and behavioral deficiencies. Hypotheses under investigation include the possibility of membrane architecture deformation, fatty acid induced mitochondrial deficiencies and disturbances in neuronal FA homeostasis. Evidence for and against these hypotheses will be presented with particular emphasis on findings linking Carnitine Palmitoyl Transferase (CPT-1) mitochondrial fatty acid import mechanisms directly to neuronal excitability. Taken together with previous evidence linking lipid peroxidation induced PLA2 activity to neurophysiological and behavioral biomarkers of neuronal aging in Lymnaea this new data substantially raises the spectre of disturbances in neuronal FFA homeostasis as an important metabolic facet of oxidative stress-dependent neuronal aging.Hermann PM, Watson SN, Wildering WC. (2014) Phospholipase A2 - nexus of aging, oxidative stress, neuronal excitability, and functional decline of the aging nervous system? Insights from a snail model system of neuronal aging and age-associated memory impairment. Front Genet. 5:419.

SA29

OXIDATIVE DNA DAMAGE AND REPAIR IN EMBRYOPATHIES AND NEURODEVELOPMENTAL DEFICITS

Peter G. Wells[1,2], Shama Bhatia[2], Danielle Drake[2]. [1]Department of Pharmacology, Toxicology, University of Toronto; [2]Faculty of Pharmacy, University of Toronto.

Birth defects and postnatal abnormalities in brain function may be caused in part by physiological or drug-enhanced embryonic and fetal levels of reactive oxygen species (ROS), which can oxidatively damage cellular macromolecules and/or alter signal transduction. Physiological ROS production alone can be developmentally toxic in genetically altered embryos and fetuses deficient in either antioxidative enzymes like glucose-6-phosphate dehydrogenase and catalase, or DNA repair enzymes/proteins like oxoguanine glycosylase 1 (OGG1) and breast cancer protein 1 (BRCA1). The enhanced developmental abnormalities in DNA repair-deficient progeny compared to wild-type (WT) littermates reveals the particular pathogenic contribution of oxidative DNA damage, likely via a non-mutagenic mechanism, as distinct from damage to other cellular macromolecules, or ROS-mediated signaling. The resulting increase in oxidative DNA damage and postnatal neurodevelopmental deficits in these deficient progeny may be relevant to the mechanisms underlying developmental abnormalities of uncertain origins, possibly including autism spectrum disorders (ASD). With in utero xenobiotic exposure, ROS formation may increased via several mechanisms, including embryonic/fetal prostaglandin H synthase (PHS)-catalyzed bioactivation of the teratogen (e.g. phenytoin, benzo[a]pyrene, thalidomide), or teratogen-initiated activation/induction of fetal ROS-forming NADPH oxidases (NOX) (e.g. methanol, ethanol, methamphetamine). Ogg1 and Brca1 knockout (KO) progeny are similarly more susceptible than WT littermates in embryo culture and/or in vivo to embryonic/fetal brain DNA oxidation, embryopathies and/or neurodevelopmental deficits caused by exposure to ROS-initiating teratogens (methamphetamine, ethanol), corroborating the pathogenic role of DNA damage, which in the case of ethanol may be relevant to fetal alcohol spectrum disorders (FASD). In response to oxidative stress, numerous protective antioxidative proteins and DNA repair proteins like OGG1 are upregulated by nuclear factor-E2-related factor 2 (Nrf2), which in turn is regulated by BRCA1. This Nrf2-dependent upregulation and protection against neurodevelopmental deficits caused by in utero exposure to methamphetamine is lost in Nrf2 KO progeny, indicating a developmentally important role for Nrf2. The increased susceptibility of BRCA1-deficient progeny also reveals a broader biological role for BRCA1, beyond cancer, in protecting the embryo/fetus from oxidative stress. (Support: CIHR)

SYMPOSIUM VIII:
Diabetes and Kidney Disease

Friday, June 3rd, 2016
10:20 AM – 10:50 AM

SA30
THE ROLE OF NOX FAMILY MEMBERS IN DIABETIC KIDNEY DISEASE
Chris R.J. Kennedy, Ottawa Hospital Research Institute, Medcine.

The NADPH oxidase (Nox) family of reactive oxygen species (ROS) generating enzymes plays an integral role in the physiology of the kidney. As our knowledge of Nox expression patterns and functions in various structures and specialized cell types within the kidney accumulates, so does the realization that Nox-derived oxidative stress contributes significantly to renal pathologies during disease progression through their ability to modify lipids and proteins, damage DNA and activate transcriptional programmes. Diverse studies demonstrate key roles for Nox-derived ROS in kidney fibrosis, particularly in settings of chronic renal disease such as diabetic nephropathy. As the most abundant Nox family member in the kidney, much emphasis has been placed on the role of Nox4 in this setting. However, an ever growing body of work continues to uncover key roles for other Nox family members, not only in diabetic kidney disease, but in a diverse array of renal pathological conditions. The objective of this seminar is to highlight the latest novel developments in renal Nox biology with an emphasis on diabetic nephropathy where oxidative stress is implicated.

SA31
ABSENCE OF TDAG51 CONFERS PROTECTION AGAINST VASCULAR CALCIFICATION
Gabriel Gyulay[1,2], Sarka Lhotak, Giusepina Pacher, Alistair Ingram, and Joan Krepinsky. [1]Hamilton Center for Kidney Research; [2]St. Joseph's Healthcare Hamilton.

Over 3 million Canadians suffer from chronic kidney disease (CKD), often leading to cardiovascular complications that significantly increase morbidity and mortality. The major pathophysiological mechanism underlying this poor prognosis in CKD is impaired vascular function due to vascular calcification (VC) in the medial layer of the vessel wall. This prominent feature of CKD is triggered by arterial smooth muscle cells (SMCs) transforming into osteoblasts, a consequence of elevated circulating phosphate levels due to poor kidney function. Understanding and targeting this SMC trans-differentiation and subsequent VC represents a viable therapeutic avenue for managing CKD complications. We have discovered that T-cell Death Associated Gene 51 (TDAG51), a protein typically upregulated by oxidative and endoplasmic reticulum stress, is induced in SMCs under conditions of hyperphosphatemia and is expressed in the medial layer of calcified human vessels. We have found that ablation of TDAG51 reduces SMC trans-differentiation and VC, both in human SMCs treated with TDAG51 siRNA and in mouse SMCs isolated from TDAG51 knockout aortas. This protection is coupled with significant changes in TGF? signalling and up-regulation of VC inhibitors. Furthermore, we have also identified a novel anti-calcific protein, Growth Differentiation Factor 10 (GDF10), which is highly expressed in TDAG51 knockout animals. Treatment of SMCs and aortic organ cultures with recombinant GDF10 protein caused a drastic reduction in phosphate-induced VC. Furthermore, we have identified that CKD patients with VC have higher circulating levels of GDF10, implicating GDF10

as a biomarker of renal and vascular injury with potential therapeutic benefits. We have established TDAG51 and GDF10 as novel mediators of VC and are further elucidating the underlying mechanisms of SMC trans-differentiation in CKD. This will enable us to better understand and eventually address this fundamentally unmet clinical need.

SA32

THE SHCD PROTEIN SENSITIZES THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) TO OXIDATIVE STRESS

Melanie K. B. Wills, and Nina Jones. Depatement of Molecular and Cellular Biology, University of Guelph.

The Epidermal Growth Factor Receptor (EGFR) is central to processes of both development and disease, and it is a known target of reactive oxygen species (ROS). ROS are emerging as important participants in both traditional growth factor-mediated cellular signalling cascades, and in non-canonical stress-induced pathways. Considering the involvement of EGFR in many cancers, efforts are underway to characterize the contributions of redox-dependent protein modifications to the mechanisms of tumourigenesis. Here, we demonstrate that the proto-oncogenic ShcD/Shc4 phosphotyrosine adaptor protein promotes stimulus-independent phosphorylation of the EGFR on three key residues of the carboxy-terminus: Y1068, Y1148, and Y1173. The response requires both the intrinsic EGFR kinase and the ShcD phosphotyrosine binding (PTB) domain. Moreover, ShcD sequesters EGFR within perinuclear vesicles and reduces the sensitivity of the cell to ligand, thereby suppressing signalling through major downstream nodes such as the MAPK Erk. Strikingly, the presence of ShcD greatly enhances oxidative stress-induced activation of the EGFR. Exposure of ShcD-positive cells to hydrogen peroxide generates more intense EGFR phosphorylation than that achieved by ShcD or ROS alone. Motifs of potential functional similarity to those within the related p66 ShcA, a known proponent of ROS, are postulated to account for this response. We therefore propose that ShcD diverts the EGFR away from canonical growth factor signalling, and instead primes it to respond to oxidative stress. These findings may have implications in cancers such as melanoma and gliomas, which have been shown to harbour increased levels of ShcD and activated EGFR.

SA33

TOLL-LIKE RECEPTORS AND NADPH OXIDASE 5 ACTIVITY; OXIDATIVE STRESS TAKES A TOLL ON RENAL FUNCTION

Chet E. Holterman[1], Naomi C. Read[1,2], Jean-Francois Thibodeau, Milica Novakovic[2], and Chris R. J. Kennedy[1,2]. [1]Kidney Research Centre, Ottawa Hospital Research Institute; [2]University of Ottawa.

Oxidative stress associated with inflammation occurs in a variety of pathological conditions including endothelial dysfunction, hypertension, chronic kidney disease, and diabetes. While the NADPH oxidase (Nox) family of ROS generating enzymes is implicated in these processes much remains to be learned about the role of the newest Nox family member, Nox5, owing partially to its absence from the mouse genome. We previously demonstrated that glomerular Nox5 expression is increased in kidney biopsies from diabetic patients and that mice with podocyte-specific transgenic expression of Nox5 (Nox5pod+) develop kidney dysfunction and are more susceptible to diabetic kidney damage. Here we demonstrate that at 12 weeks of age Nox5pod+ animals have higher basal glomerular inflammation and increased glomerular expression of Toll-like Receptors (TLRs) and pro-inflammatory cytokines IL-1β and IL-6 suggesting that Nox5 may play a role in TLR signalling and inflammation. In a lipopolysaccharide (LPS) model of acute kidney injury (AKI) both Nox5pod+ and non-TG animals treated with LPS showed increased TLR receptor and Nox1 expression. However compared to non-TG animals, Nox5pod+ animals had significantly greater expression of IL-1β and

a corresponding increase in infiltrating macrophages in the kidney cortex as well as an increase in glomerular inflammation and filtration barrier dysfunction. To further delineate the molecular mechanism we exposed conditionally-immortalized human podocytes (hPODs) to LPS and found significant increases in TLRs, Nox5, and inflammatory cytokine expression. LPS stimulation of hPODs resulted in a significant increase in ROS production that could be blocked by inhibition of interleukin-1 receptor-associated kinases -1 and -4 (IRAK1/4) that lie immediately downstream of the TLRs. Co-immunoprecipitation assays demonstrated a direct interaction between Nox5 and IRAK1. Furthermore, immunoprecipitation of Nox5 from LPS-treated hPODs and subsequent western blotting demonstrated an increase in Nox5 phospho-threonine that could be blocked by pre-incubation with the IRAK1/4 inhibitor. These results are the first to demonstrate that Nox5 is a direct downstream target of TLRs and highlight a role for Nox5-derived ROS in driving a pro-inflammatory state via the pathogen and damage sensing TLR pathway.

SYMPOSIUM IX:
Transcriptional Control and Immunology

Friday, June 3rd, 2016
1:10 PM – 2:30 PM

SA34
REGULATION OF NFE2L3 (NRF3) TRANSCRIPTION FACTOR DEGRADATION BY FBW7 AND GSK3
Volker Blank, Meenakshi Kannan, and Isadore Dodard-Friedman. Lady Davis Institute for Medical Research/McGill University.

The NFE2L3 transcription factor has been implicated in various cellular processes, including carcinogens, stress response, differentiation and inflammation. NFE2L3 forms heterodimers with small Maf proteins and binds antioxidant response element (ARE) consensus sequences, found in the regulatory regions of detoxification enzyme genes. In previous studies, we have shown that NFE2L3 exhibits a rapid turnover and is stabilized by proteasomal inhibitors. We thus investigated the mechanisms involved in the degradation of this transcription factor. We found that NFE2L3 is ubiquitinated and that its degradation is mediated by the E3 ubiquitin ligase FBW7. We showed that FBW7 interacts with NFE2L3 and that dimerization of FBW7 is required for degradation of the transcription factor. We also demonstrated that NFE2L3 is phosphorylated by GSK3 and found that this kinase mediates the FBW7-dependent ubiquitination of NFE2L3. We also showed that FBWX7 abrogates the NFE2L3-mediated repression of the ARE of the NAD(P)H:quinone oxidoreductase 1 (NQO1) gene, coding for a detoxification enzyme. Our findings reveal FBW7 and GSK3 as novel regulators of the NFE2L3 transcription factor and a potential mechanism by which FBW7 might regulate detoxification and the cellular response to stress.

SA35
NRF1: THE LESSER-KNOWN PLAYER IN THE ANTIOXIDANT RESPONSE
William Willmore, Institute of Biochemistry, Departments of Biology and Chemistry, Carleton University.

The Antioxidant Response is the activation of gene expression in response to various stresses including oxidative stress, xenobiotic stress and inflammation. These responses involve the activation of a DNA element known as the Antioxidant Response Element (ARE) or Electrophile Response Element (EpRE). The factors that bind to AREs/EpREs include the Nuclear Factor (Erythroid-derived 2)-Like proteins (NFE2L or Nrf) and the small Maf proteins. Nrf is a member of the Cap'n'Collar (CNC) family of basic-region leucine zipper (bZIP) transcription factors (Nrf1, Nrf2 and Nrf3) and activate gene expression from AREs/EpREs found in the promoter and enhancer regions of the genes for antioxidant enzymes, enzymes of xenobiotic metabolism and inflammatory factors. In comparison to Nrf2, there are relatively fewer studies on Nrf1, in comparison to Nrf2. This may be due to a) their very different mechanisms by which they are regulated, b) the knockout mouse for Nrf2 is completely viable, whereas the knockout mouse for Nrf1 is embryonic lethal and c) their different locations within the cell (Nrf2 is cytosolic, whereas Nrf1 is bound to the endoplasmic reticulum (ER)). With this talk, the differences in the CNC family members will be outlined, and data will be presented

on their responses to various stresses (oxidative stress, hypoxia, ER stress and toxin stresses). An overview will be provided on "cross-talk" between these stresses, effected through the common factor of Nrf1. The implications of such stress cross-talk, in the response to the individual stresses, will be discussed.

SA36

DOES THE DNA METHYLATION IS DEPENDENT ON THE REDOX POTENTIAL VALUE OF GLUTATHIONE?

Jean-Claude Lavoie, Wesam Elremaly, Thérèse Rouleau. Department of Nutrition, CHU Sainte-Justine, Université de Montréal.

The origin of several health complications observed in adults born <30 weeks of gestation, dates back to their first weeks of life; epigenetic modifications are suspected. The parenteral nutrition (PN) of these preterm infants, which is contaminated with peroxides, induces an oxidation of the redox potential of glutathione in blood. Methylation of DNA depends on the availability of the substrate S-adenosylmethionine (SAM), produced by the methionine adenosyltransferase (MAT), and the activity of DNA methyltransferases. Animals infused with PN or H2O2 at concentration measured in PN, present a decrease in MAT activity and DNA methylation. The oxidation of a specific cysteinyl residue limits the activity of MAT. The recycling of the oxidized thiol is dependent of glutathione availability and value of redox potential. **Hypothesis**: The redox potential regulates the methylation of DNA. **Objective**: To validate correlation between MAT activity and redox potential in liver of newborn guinea pigs. **Methods**: Since their third day of life, guinea pigs were infused through a catheter in jugular vein with solutions designed to obtain different values of redox potential: 1) dextrose, 2) 350µM H2O2 (as in PN), 3) 180µM ascorbylperoxide (present in PN), 4) PN (amino acids, dextrose, lipids, vitamins), 5) PN + glutathione and 6) Sham (animals with closed catheter, fed by mouth). After 4 days, MAT activity (U = nmol/min/mg prot) and redox potential (mV) were determined in liver. **Results:** Correlations were significant between MAT and redox potential according to the nutritional status of the animal. In PN groups, the correlation was ($y = a \cdot x + b$) $-0.032U \cdot mV^{-1} \cdot x - 5.3U$; $r^2 = 0.56$, $p<0.01$, $n=14$). In animals without PN, the correlation was $-0.080U \cdot mV^{-1} \cdot x - 14.3U$; $r^2 = 0.70$, $p<0.01$, $n=22$). The slopes were different ($p<0.01$). **Conclusion:** More oxidized is the redox potential lower is the activity of MAT. Independently of redox potential, PN inhibits MAT. Because the level of SAM in vivo is close to the Km of DNMT, a modification in the activity of MAT has inevitably an impact on SAM concentration. Therefore, redox potential of glutathione is a possible regulator of the DNA methylation.

SA37

ANTI-MPO PRODUCED FROM MPO-BASED PROTEIN FREE RADICALS. IMPLICATIONS FOR DRUG-INDUCED AGRAULOCYTOSIS& LUPUS

Arno Siraki, Deaprtment of Pharmacy and Pharmaceutical Sciences, University of Alberta.

The objective of this study was to assess if murine myeloperoxidase (MPO) can trigger an immune response in mice upon aminogluthethimide (AG) metabolism. AG is a drug that was widely used in the past to treat estrogen-dependent breast cancer. However, its use was hampered by an approximate 1% incidence of agranulocytosis – the depletion of neutrophils to very low levels that can lead to infection and sepsis. Previous studies by our group have shown that AG metabolism by human MPO (the dominant heme protein found in neutrophils) resulted in protein radical formation. However, the biological relevance of this type of post-translational modification is unknown. We hypothesized that the modification would be recognized as non-self by the murine immune system and would trigger an immune response. To produce MPO protein free radicals, we carried out reactions previously established to produce MPO protein radicals that were initially detected by using immuno-spin trapping (using DMPO). The conditions to detect the MPO-protein radical required the presence of AG, H2O2, and DMPO (to trap the protein radical). Detection was carried out using anti-DMPO and Western blotting. Control reactions (i.e., without H2O2, or AG) were not recognized with anti-DMPO. Once the conditions were optimized, reactions were prepared in the absence of DMPO in order to produce the putative immunogen. The reactions were dialyzed two time and sterile filtered before administration to mice. Mice were divided into 4 groups: control (untreated), AG+MPO, H2O2+MPO, and AG+H2O2+MPO. Mice were administered 10 ug of MPO in each treatment group (n=7). A standard immunization protocols was applied that utilized Freund's complete adjuvant and incomplete Freund's adjuvant. Sera were collected at day 36, and were analyzed for anti-MPO antibodies using a commercial ELISA for murine anti-MPO detection and were further confirmed by Western blot. Only sera from mice treated with AG+MPO+H2O2 only demonstrated significant concentrations of circulating anti-MPO. These results shed light into the biological importance of AG-dependent free radical formation on MPO. Further studies will be directed towards generating the immunogen in vivo.