Poster Presentation Abstracts

Wednesday, June 1st, 2016: Odd Number Posters Presentation
Thursday, June 2nd, 2016 Even Number Posters Presentation
12:30PM – 2:00 PM
Location: Rozanksi Concourse

PPA01
EFFECTS OF OXIDATIVE STRESS AND EXTRACELLULAR HEMOGLOBIN OR ITS DEGRADATION PRODUCTS ON MYELIN COMPONENTS: POTENTIAL ROLE IN MS PATHOGENESIS.

Vladimir V. Bamm, Danielle K. Lanthier, Shannon L.J. Sproul, and George Harauz.Molecular and Cellular Biology, University of Guelph.

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS). There is a well-documented relationship between cerebral vasculature and MS lesions: abnormal accumulations of iron have been found in the walls of the dilated veins in cerebral MS plaques. The source of this iron is unknown, but capillary and venous hemorrhages leading to blood extravasation have been previously recorded. In turn, hemorrhaging leading to hemolysis results in release of hemoglobin extracellularly, which is a reactive molecule that could lead to local oxidative stress, inflammation, and tissue damage. Our previous studies with a reduced form of hemoglobin (oxyHb) have demonstrated its ability to cause extensive lipid and protein oxidation in vitro, which would result in membrane destabilization.Here, we investigate whether a more abundant oxidized form of extracellular hemoglobin (metHb) and dissociated hemin can cause direct oxidative damage to myelin components in vitro and, thus, play a role in the progression of MS. Specifically, we use membrane-mimetic lipid vesicles and myelin basic protein (MBP), a highly abundant protein in the CNS, to analyze oxidative products and elucidate the mechanism of oxidative damage. Oxidation of lipids was assessed by the formation of conjugated diene/triene and malondialdehyde, and oxidation of MBP was analyzed by the change in protein mass. The effects of metHb and hemin on cell differentiation and myelination were also studied using the murine oligodendrocyte precursor cell line OliNeu. Our preliminary results show that metHb causes oxidative damage to MBP and myelin lipids, potentially by transferring its hemin moiety to protein and lipid. The addition of exogenous H2O2 (to mimic the inflammatory environment) increases the oxidation potential of metHb, possibly via redox reaction of the dissociated hemin or formation of a ferryl radical.This study is expected to uncover the mechanism of extracellular hemoglobin-induced oxidative damage to myelin components and shed light on new potential events in MS pathogenesis. Our results support further research into the vascular pathology in MS, to gain insight into the role of iron deposits in MS pathogenesis and/or in stimulation of different comorbidities associated with the disease.

PPA02

EPIGENETIC MECHANISMS MAY CONTRIBUTE TO POSTNATAL NEURODEVELOPMENTAL ABNORMALITIES IN UNTREATED AND ETHANOL-EXPOSED DNA REPAIR-DEFICIENT OXOGUANINE GLYCOSYLASE 1 (OGG1) KNOCKOUT PROGENY

Shama Bhatia[1], and Peter G. Wells[2]. [1]Deparmentof Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto; [2]Deparment of Pharmacology; Toxicology, Faculty of Medicine, University of Toronto.

Oxidative DNA damage and altered DNA methylation occurs in the brains of autistic mouse models, and in the fetal brains of mice exposed in utero to ethanol (EtOH). We previously reported that reactive oxygen species (ROS)-initiated DNA damage in fetal brains, and postnatal neurodevelopmental deficits, were enhanced in EtOH-exposed oxoguanine glycosylase 1 (Ogg1) knockout (KO) progeny, which cannot repair the pathogenic DNA lesion 8-oxo-2'-deoxyguanosine lesion (8-oxodG). Using Ogg1 KO mice, we are investigating the role of 8-oxodG-dependent epigenetic changes, particularly 5-methylcytosine (5-mC) formation, in the mechanism of postnatal neurodevelopmental abnormalities initiated by physiological and EtOH-enhanced levels of ROS, potentially relevant to autism and fetal alcohol spectrum disorders (ASD, FASD) respectively. 5-mC in the brains of untreated young and adult Ogg1 wild-type (WT) and KO progeny and in EtOH-treated Ogg1 WT and KO fetal brains exposed in utero was measured by ELISA, and repetitive behaviour for young mice by a marble burying test. Brain 5-mC was not different in young Ogg1 KO vs. WT mice, but was increased in adult Ogg1 KO mice vs. young Ogg1 KO mice (p<0.05), showing an increase with age. Postnatal repetitive behaviour was increased in Ogg1 KO mice vs. Ogg1 WT mice (p<0.001) in male but not female progeny. The effect of ROS-mediated 8-oxodG levels on 5-mC was measured in fetal brains extracted 1 and 6 h post maternal treatment with EtOH (2 g/kg i.p.) on gestational day 17. Fetal brain 5-mC was increased at 6 h in EtOH-treated Ogg1 WT progeny (p<0.01) but not in EtOH-treated Ogg1 KO littermates, compared to saline-treated Ogg1 WT and KO progeny respectively. The results suggest that inability to repair 8-oxodG can result in an increase in 5-mC with age, which may contribute to the neurodevelopmental abnormalities seen in untreated Ogg1 KO mice. A similar increase in 8-oxodG and 5-mC levels, with a decrease in Ogg1 expression, has been reported in autistic mouse models. Neurodevelopmental abnormalities in DNA repair-deficient progeny caused by physiological and EtOH-enhanced ROS formation may be mediated in part by DNA methylation, and OGG1 may be a biomarker of risk for ASD and FASD. (Support: CIHR).

PPA03

NANOSILVER INDUCTION OF CELLULAR OXIDATIVE STRESS RESPONSE AND DETOXIFICATION PATHWAYS

Shana Cameron[1], William Willmore[1], Farah Hosseinian[2], and Owen Hovey[1]. [1]Department of Chemistry, Carleton University; [2]Department of Biology, Carleton University.Institute of Biochemistry, Carleton University.

Nanosilver (1-100 nm silver particles) has antimicrobial properties and is widely used in many commercial, engineering, and biomedical applications. The potential effects of direct nanosilver exposure to human cells and other organisms through consumer products is largely unknown. Due to the increased use of nanosilver in these products, exposure to this nanomaterial is increasing. Thus, it is crucial to understand the potential

biological effects and mechanistic perturbations caused by nanosilver at the cellular level.This novel research examines the relative induction of the two primary detoxification pathways upon exposure to nanosilver. The first pathway is the aryl hydrocarbon receptor, which induces various cytochrome P450s through the xenobiotic response element (XRE). The second pathway is the nuclear factor-erythroid 2 p45 subunit-related factors, which induce various xenobiotic-metabolizing and antioxidant enzymes through the electrophile response element/antioxidant response element (EpRE/ARE). Nanosilver was found to effect the production of reactive oxygen species (ROS), as well as to cause depletion of the major cellular antioxidant molecule glutathione. Additionally, cell cycle arrest was observed, with an increased number of cells accumulating in the G1 and S phase for the nanosilver and AgNO3 treated cells, respectively. This research provides new information and insight into the mechanisms and pathways affected by nanosilver on the cellular level.

PPA04

INNATE DEFICITS IN DENDRITIC OUTGROWTH IN PARKINSON'S PATIENT-DERIVED NEURONS ARE RESCUED BY NRF2-MEDIATE ACTIVATION OF THE ANTI-OXIDANT RESPONSE

Chris Czaniecki, Arianne Cohen, Juliane Heide and Scott D. Ryan, University of Guelph

Parkinson's Disease (PD) is associated with pathological deposits of aggregated α-synuclein (α syn) in multiple brain regions. While motor dysfunction is a primary phenotype, many patients suffer from cognitive impairment including dementia. PD dementia is highly associated with pathological deposits of aggregated α syn in neurites. Much evidence suggests that increased Reactive Oxygen/Nitrogen Species (ROS/RNS), resulting from impaired mitochondrial function, contribute to this pathology. However, the mechanism of neuronal loss in PD remains unclear. We utilized a patient-derived hiPSC model of PD that allows for comparison of A53T α syn mutant neurons against isogenic corrected controls, in addition to a hESC model with the A53T α syn mutation introduced. Following differentiation to dopaminergic (DA) neurons, we determined that A53T DA neurons display reduced neurite outgrowth and diminished neuritic complexity. Strikingly, neuritic morphology of A53T DA neurons was rescued by treatment with L-NAME, a nitric oxide synthase inhibitor, suggesting a causal link to nitrosative stress. Furthermore, we show that activation of NRF2, a master regulator of the antioxidant response, normalizes neurite outgrowth. Our results reveal innate differences in neuritic morphology between PD-A53T DA neurons and isogenic controls. More importantly, we show that this phenotype can be rescued through the alleviation of nitrosative stress. As such, detoxifying neurons of ROS/RNS through forced activation of NRF2 may provide a new therapeutic avenue against PD and associated dementia.

PPA05

LOCALIZED CONTROL OF OXIDIZED RNA

James Dhaliwal[1], Yu Zhan[1], PaulineAdjibade[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 (ROS) can render them inactive or toxic.This includes the oxidation of RNA, which appears to underlie the detrimental effects of oxidative stress, aging and certain neurodegenerative diseases. Here, we investigate themanagement of oxidized RNA in the chloroplast of the green alga Chlamydomonas reinhardtii. Our immunofluorescence microscopy results reveal that oxidized RNA (with 8-hydroxyguanine) is 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 (RBCL), but not Rubisco, in the management of oxidized RNA. An RBCL pool that can 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 that 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.

PPA06

METABOLIC CHANGES DURING EXTRAEMBRYONIC ENDODERM DIFFERENTIATION

Mohamed Gatie, Department of Biology, Western University

During mouse early embryogenesis the primitive endoderm (PrE) forms from cells originating from the inner cell mass. This is recapitulated in vitro using F9 teratocarcinoma cells, which on exposure to retinoic acid (RA) or WNT6 form PrE, and with the subsequent addition of dibutyryl cyclic AMP (db-cAMP), they differentiate into parietal endoderm (PE). During this differentiation the cells switch from glycolytic metabolism towards oxidative phosphorylation. We have shown that F9 cells treated with RA or RA and db-cAMP show reduction in the transcript and protein levels of glycolytic markers such as PKM1/2, LDHA, PDK1, and phospho-PDH. Concomitantly, we see increased mitochondrial activity and mitochondrial ROS production. We have also shown that H2O2 can induce the differentiation of F9 cells towards PrE by activating the canonical Wnt/β-catenin pathway. In contrast, when F9 cells were treated with rotenone (complex I inhibitor) differentiation is attenuated, suggesting that oxidative phosphorylation is required for F9 cell differentiation. Based on these results we are proposing that the switch from glycolysis towards oxidative phosphorylation and subsequent increase in mitochondrial activity and ROS production, precedes the changes in Wnt-dependent gene expression that are required for differentiation of the primitive endoderm.

PPA07

DETECTION OF OXIDATIVE STRESS SIGNATURES IN LIVING CELLS OF THE GREEN ALGA CHLAMYDOMONAS REINHARDTII

Kira Goff[1], Tom Ellis[2], and Kenneth Wilson[2]. [1]Department of Biology, University of Saskatchewan; [2]Department of Chemistry, University of Saskatchewan.

Photosynthetic organisms are especially prone to oxidative stress, given the nature of the reactions used to convert light energy to chemical energy. In particular, the presence of chlorophyll as the primary light absorbing pigment, leads to the generation of singlet oxygen if there is a disruption in the electron transport chain. Work on model plants and algae has begun to unravel a signaling and response system, with transcription of specific genes modified as a result of singlet oxygen stress. However, far less is known about the biochemical response of individual living cells. In this study, we use a combination of high-light exposure and chemical photosensitizers to induce singlet oxygen stress in cells of the green alga Chlamydomonas reinhardtii. To study the effects in vivo, we use synchrotron-based Fourier Transform Infrared Spectromicroscopy (FTIR). By comparing absorbance bands known to correspond to lipids, proteins, and DNA, damage and repair mechanisms can be observed. To better understand the impacts of singlet oxygen mediated stress on individual cells we used a set of photosynthetic mutants with varying sensitivities to high light exposure. We found that biochemical changes in living cells were dependent upon both photosynthetic mutation and route of oxidative stress exposure. Our measurements could differentiate between cells exposed to high-light induced oxidative stress, chemically-induced oxidative stress, or a combination of both. The

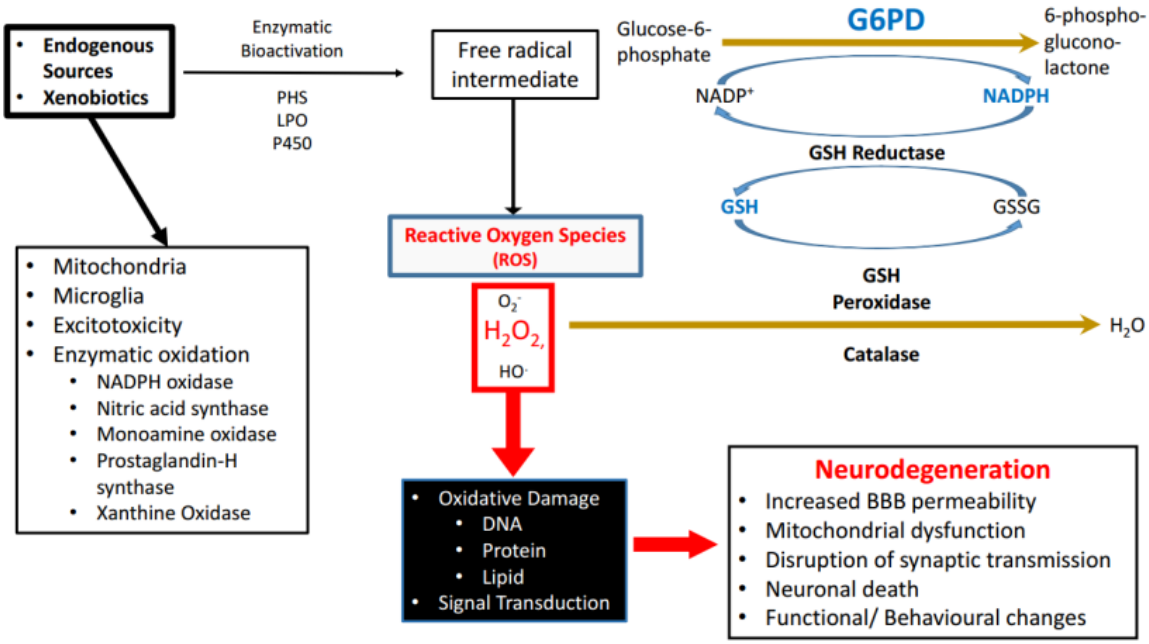
different sources of stress induction produced different biophysical response signatures. The strongest changes were observed in bands relating to protein secondary structure and lipid configuration. Strikingly, FTIR of lipid-associated CH2 and CH3 bands indicated a phase transition amongst membrane lipids and increased membrane disorder as a result of oxidative stress. This was supported by increased hydrogen-deuterium exchange across the membrane and the conversion of the amide II protein band into the amide II prime (hydrogen-deuterium) protein band. These biochemical changes correlated with visible cell expansion. Membrane changes were most pronounced in the presence of chemical photosensitizers, though they were also found amongst some photosynthetic mutants during time-resolved studies of in situ high light exposure in individual cells.

PPA08

BEHAVIOURAL NEURODEGENERATIVE OUTCOMES AND GENDER-DEPENDENT BRAIN DNA DAMAGE ARE EXHIBITED IN YOUNG AND AGING MUTANT MICE DEFICIENT IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Anmol Gupta[1], Shama Bhatia[1], Isabel Mackay-Clackett[2], and Peter G. Wells[2], [1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto; [2]Department of Pharmacology and Toxicology, Faculty of Medicine, University of Toronto.

Aging is associated with neurodegenerative disorders potentially mediated in part by the accumulation of oxidative macromolecular damage caused by reactive oxygen species (ROS), particularly with oxidative stress resulting from an imbalance in the formation and detoxification of ROS. Even in the absence of ROS-inducing xenobiotics, physiological levels of ROS production can become pathogenic when antioxidative or DNA repair pathways are compromised, as observed in mice deficient in glucose-6-phosphate dehydrogenase (G6PD), which provides NADPH necessary for maintaining reduced glutathione. Previous studies revealed that the brains of aging, mutant G6PD-deficient mice show increased DNA oxidation and pathological cellular changes. To determine the functional consequences of these molecular and cellular neurodegenerative changes, we evaluated young (2-5 months) and aging (12-18 months) male and female G6PD-deficient mice for brain DNA damage (γH2AX reflecting DNA double strand breaks) and measures of brain function (puzzle box for executive function) and social dominance (tube test). In the puzzle box test, both young and aging female wild-type (+/+)G6PD mice variably performed better than G6PD-deficient (def/def) controls ($p<0.05$). In contrast, a lesser but opposite trend was seen in the males, where G6PD-deficient (def/y) mice performed better than male wild-type (+/y) G6PD-normal controls in a single trial ($p<0.05$). In the tube test for social dominance, among young mice, both male and female G6PD wild-type mice won more unique matchups than their gender-matched G6PD-deficient opponents. DNA damage varied by age, gender and specific brain region, including respectively increased hippocampal and cerebellar γH2AX in young and aging female def/def G6PD mice, and similarly increased hippocampal γH2AX in aging male def/y mice ($p<0.05$). In contrast, young male def/y mice exhibited decreased striatal, hippocampal and cerebellar γH2AX levels, consistent with their better puzzle box performance. Overall, these results are consistent with the molecular and cellular brain damage previously reported in aging G6PD-deficient mice, and provide the first evidence of functional neurodegenerative consequences.DNA damage appears to accumulate in the brains of G6PD-deficient mice from an early age and causes subtle, gender- and age-dependent aberrations in brain function, suggesting a role for G6PD in protecting against ROS-mediated neurodegenerative diseases (Support: CIHR).



PPA09

MITOCHONDRIAL BIOENERGETIC DYSFUNCTIONS IN HUMAN TYPE 1 DIABETIC SKELETAL MUSCLE?

Meghan C Hughes[1], Sofhia V Ramos[1], Donna D'Souza[2],Thomas J Hawke[2], and Christopher GR Perry[1].[1]Muscle Health Research Center, School of Kinesiology and Health Science, York University; [2]Department of Pathology and Molecular Medicine, McMaster University.

In Type 1 Diabetes, appropriate management of glycaemia significantly improves risk factors for several co-morbidities including cardiovascular and renal disease. However, emerging evidence in rodent models indicate a previously unrecognized myopathy within skeletal muscle may existing relation to myogenesis and metabolic function. Given the paucity of information in humans, we hypothesized that an unrecognized mitochondrial pathology exists across a spectrum of bioenergetic functions in human skeletal muscle from Type 1 Diabetics. In this exploratory pilot experiment, we recruited Type 1 Diabetic (T1D; 2 male, 1 female) and healthy Control participants (1 male, 2 female) aged 27.5+/-0.3 years. Only 1 T1D exhibited a high level of habitual physical activity. Each participant received a percutaneous microbiopsy of the vastus lateralis while lying at rest. Muscle was used to prepare permeabilized fibre bundles to assess mitochondrial bioenergetics*in vitro*. Notably, complex III-derived H_2O_2 emission (antimycin A) was significantly elevated in T1D (8.1+/-0.6 vs Control: 3.9+/-0.9 pmol/min/mg dry wt, p=0.04). Pyruvate-supported State 3 respiration (ADP-stimulated) as an index of carbohydrate oxidation was lower in T1D (24+/-2.9 vs Control: 36.5+/-1.6 pmol O_2/sec/mg wet wt, p=0.048 @ 10mM ADP stimulation). Of particular interest, the participant with the most well-managed glycaemia (insulin pump) and high degree of physical activity demonstrated the lowest rate of H_2O_2 emission in 3 stimulation protocols (glycerol 3-phosphate dehydrogenase, succinate-supported Complex I, and pyruvate/malate-supported Complex I).Likewise, this same T1D participant demonstrated a calcium retention capacity - an index of susceptibility to mitochondrial permeability transition-induced apoptosis - that was similar to Control (12.9 vs Control: 9.2 +/- 1.3 nmol Ca^{2+}/mg dry wt). Conversely, the 2 remaining T1Ds with poorly managed glycaemia/light physical activity demonstrated very low retention capacities (0.6-0.7 nmol Ca^{2+}/mg dry wt). This initial examination raises the intriguing possibility that young adults with Type 1 Diabetes may have a previously unrecognized skeletal muscle deficiency in mitochondrial

bioenergetics. Furthermore, the case of a T1D with well-managed glycaemia and high level of physical activity has led to our current hypothesis that potential mitochondrial deficits can be corrected with combinatorial intensive insulin and exercise therapies in young adulthood.

PPA10

CARDIOLIPIN FACILITATES REFOLDING OF A-SYNUCLEIN FIBRILS IN RESPONSE TO ACCUMULATION OF MITOCHONDRIAL STRESS

Kayla M. Humphries, Vladimir V. Bamm, Carla L. Coackley, George Harauz, and Scott D. Ryan. Department of Molecular and Cellular Biology, The University of Guelph.

Neuronal loss in Parkinson's disease (PD) is associated with aberrant mitochondrial function and impaired proteostasis in dopaminergic neurons. Linking these two pathologies is a major hurdle in developing new therapies for PD. Using neurons exposed to either pre-formed ?-synuclein fibrils (PFFs) or the mitochondrial complex I inhibitor rotenone, we determined that both exposures result in rapid accumulation of mitochondrial superoxide anion, coupled to externalization of the anionic phospholipid, cardiolipin, from the inner mitochondrial membrane (IMM) to the outer mitochondrial membrane (OMM). To explore the link between cardiolipin externalization and synucleinopathy, we exposed unilamellar lipid vesicles (LUVs) with increasing molar ratios of cardiolipin (to mimic the composition of the OMM) to PFFs, and determined the effect on both -syn binding and protein conformation. We found that cardiolipin specifically bound to and facilitated re-folding of -syn fibrils. Moreover, we found that ?-syn harboring the A53T disease-causing mutation required higher concentrations of cardiolipin and longer exposure times to facilitate the same degree of re-folding. These results suggest a link between mitochondrial stress events, accumulation of reactive oxygen species, and synucleinopathy in PD.

PPA11

OXIDIZED VITAMIN C CATALYZED S-HOMOCYSTEINYLATION

GraceAhuie Kouakou[1], Hicham Berrougui[2], Abdelouahed Khalil[2], and Klaus Klarskov. [1]Université de Sherbrooke; [2]Department for Medicine and Geriatrics;

Vitamin C (AA, ascorbic acid; ascorbate ion at physiological pH) is a well-known oxidative stress scavenger of organisms. In this process, ascorbate is oxidized by the loss of one electron to give initially an ascorbyl free radical (SDA), which subsequently undergoes oxidation to dehydroascorbate (DHA). Under physiological conditions, both SDA and DHA are recycled back to ascorbate through the action of several enzymes including NADH-cytochrome b5 reductase, thioredoxin reductase, protein disulfide isomerase, glutaredoxin-1 (thioltransferase), and certain glutathione-S-transferases. Homocysteine (Hcy) is an amino acid that is not incorporated into the amino acid sequence of peptides and proteins. Hcy is produced from methionine and is a homologue of cysteine that differs by an additional methylene bridge. A high blood level of homocysteine (Hcy) is a recognized risk factor for several major oxidative stress related pathologies such as cardiovascular diseases and neurological diseases. However, the mechanism(s) by which hyperhomocysteinemia induces endothelial and neurological dysfunction is still unknown. Since Hcy efficiently reduces DHA to AA, we investigated if in this process DHA induces peptide and protein S-homocysteinylation. With cysteine containing peptides we demonstrate that DHA catalyses S-homocysteinylation as a function of time, indicating a possible direct link between oxidative stress represented by DHA and homocysteine. Using a combination of tandem mass spectrometry coupled with liquid chromatography, we show that DHA also

catalyses S-homocysteinylation of reduced insulin and paraoxonase-1. The latter protein is associated with the high-density lipoprotein complex and has been suggested to play a role in its protection against oxidative stress. The biological importance of these modifications remains to be elucidated.

PPA12

THE ADAPTOR PROTEIN P66SHC REGULATES METABOLISM, ROS PRODUCTION AND AMYLOID BETA SENSITIVITY IN CNS CELLS

Asad Lone,Western University.

A key pathological feature of Alzheimer's disease (AD) is the accumulation of extracellular deposits of amyloid beta (Aß) peptide within the brains of affected individuals. Aß accumulation is associated with oxidative stress, extensive neuronal death and synaptic loss. However, 40% of the elderly have pronounced Aß deposition within their brains, yet show no symptoms of dementia, indicating that some cells are resistant to Aß toxicity. Several studies suggest that central nervous system (CNS) cells that are resistant to the harmful effects of Aß display a metabolic shift from mitochondrial-dependent oxidative phosphorylation (OXPHOS) to aerobic glycolysis for their energy needs. The adaptor protein p66SHC has been shown to play a definitive role in aging, as well as in regulation of redox balance and ROS levels. Recent studies have shown that p66SHC expression and activation can shift the cellular metabolic state from OXPHOS to aerobic glycolysis. Hence, we propose that the expression and activation of p66SHC in CNS cells promotes both increased OXPHOS and sensitivity to Aß toxicity. To test this hypothesis we transiently overexpressed p66SHC in a rodent neuronal cell line, and knocked down endogenous p66SHC in a rodent glial cell line, to determine the effect of p66Shc activation on metabolic activity. Changes in mitochondrial ROS levels and mitochondrial electron transport chain (ETC) activity were also measured. We examined the subcellular localization of the phosphorylated and activated form of p66SHC. Lastly, we investigated if p66SHC expression and activation affected sensitivity to Aß in both neuronal and glial cell lines in an OXPHOS dependent manner. Transient overexpression of p66SHC repressed glycolytic enzyme expression and increased mitochondrial ETC activity and ROS levels. The opposite effect was observed when endogenous p66SHC expression was knocked down. Exposure to Aß promoted the phosphorylation and activation of p66SHC, resulting in an upregulation of mitochondrial metabolism. Moreover, expression and activation of p66SHC increased sensitivity to Aß toxicity. Our findings indicate that expression and activation of p66SHC renders CNS cells more sensitive to Aß toxicity by promoting mitochondrial OXPHOS while repressing aerobic glycolysis. Thus, p66Shc may represent a therapeutically relevant target for the treatment of AD.

PPA13

LOSS OF 5-HYDROXYMETHYLCYTOSINE IN BRAIN TUMORS RELATED TO VARIATIONS IN EPIGENETIC MARKS.

Carolyne Mary Lowry, J. Richard Wagner, Laurent-Olivier Roy, David Fortin, and Marie-Belle Poirier. Université de Sherbrooke.

Of the wide range of brain tumors that form in the human brain, glioblastoma multiform (GBM) is the most common tumor detected. GBM is considered as a grade IV astrocytoma, where the median survival rate for patients rarely is about 16 months. About 90% of GBMs develop rapidly and are considered to be primary or de novo, whereas the remainder begin at an earlier stage and grow into secondary GBMs. Causes for GBMs

are widely unknown. Numerous studies suggest that epigenetic factors are closely associated with the development and growth of gliomas. A prominent epigenetic mark in the genome arises from the methylation of cytosine by DNA methyltransferase creating 5-methylcytosine (5mC). Recently, an additional epigenetic mark was discovered involving the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) by TET family enzymes. This may be an initial step in the pathway to remove 5mC from the genome. The levels of 5hmC have been shown to be higher in the brain than in any other tissue. However, during the development and growth of brain tumors, these levels dramatically decrease. The mechanisms implicated in the reduction of these levels are still uncertain. The goal of this study is to ascertain why the levels of 5hmC are low in brain tumors. Using LC-MS/MS analysis, we monitored the levels of 5mC and the corresponding oxidation products (5hmC, 5fC, 5hmU 5fU) within the DNA of glioma tumor specimens obtained from surgery. From the analysis of 110 brain tumors, we observed that the levels of 5hmC were reduced by as much as 40-fold in tumor compared to normal brain tissue. In contrast, the levels of 5hmC were unchanged. Furthermore, we observed that the levels of 5hmC were lower within primary GBMs than in grade II/III gliomas including secondary GBM. Positive correlations were noted between 5mC and 5hmC within primary GBM, but also between 5mC and 5fC in grade II/III glioma and secondary GBM. These results support previous studies attesting to the low levels of 5hmC in brain tumors, but also highlight possible pathways that may explain the abnormal removal of 5hmC from the tumor genome.

PPA14

4-PBA TREATED DRINKING WATER SLOWS ATHEROSCLEROTIC LESION GROWTH IN APOE-/- MICE

Edward G. Lynn[1,2], Šárka Lhoták, Richard C. Austin. [1]Department of Medicine, Division of Nephrology, McMaster University; [2]St. Joseph's Healthcare Hamilton.

Cardiovascular disease (CVD) and its complications, such as plaque rupture, thrombus formation and vessel occlusion, account for the majority deaths worldwide. The infiltration of monocytes and their differentiation to macrophages are key events in atherogenesis. Previously we have shown that the unfolded protein response (UPR) has an important role in monocyte-macrophage differentiation. Further, endoplasmic reticulum (ER) stress-dependent apoptosis contributes to lesion progression and plaque necrosis. The aim of the current study was to determine whether 4-phenylbutyrate (4-PBA), a small chemical chaperone that alleviates ER stress, provides protection versus atherosclerosis when administered via drinking water. Female Apoe-/- mice on chow diet were provided 4-PBA (2.5g/kg/d) ad libitum for five weeks starting at 12 weeks of age. Early atherosclerotic lesion area was significantly reduced in 4-PBA treated Apoe-/- mice, relative to controls (0.6 x105 ± 0.1 ?m2 versus 1.1 x105 ± 0.1 ?m2, respectively, P < 0.05) as measured by immunohistochemistry. We hypothesized that 4-PBA reduced lesion size, in part, through a mechanism involving the alleviation of ER stress and decreased monocyte-macrophage differentiation. In support of this hypothesis, 4-PBA inhibited monocyte-macrophage differentiation, as measured by mRNA expression of the macrophage marker MSR-1 (0.5 ± 0.2 fold-change vs. control) and cell adherence in PMA-treated THP-1 cells (37 ± 5% vs. control). Nanostring and qRT-PCR analyses of RNA isolated from Apoe-/- peritoneal macrophages indicated 4-PBA treatment induced expression of HSP25/27, a small heat shock protein family member previously shown to be protective against lesion growth in mouse models, and positively associated with reduced CVD in patients. In summary, these data provide evidence that 4-PBA treated drinking water delayed atherosclerotic lesion growth in a mouse model of atherosclerosis. We postulate that 4-PBA acts via multiple mechanisms, including reduction of ER stress, modulation of monocyte-macrophage differentiation

as well as HSP25/27 expression. Additional studies are in progress to further delineate the mechanisms by which 4-PBA affects atherosclerosis.

PPA15

ISGYLATION IS A HYPOXIA-INDUCED PATHWAY THAT ATTENUATES ACTIVITY OF THE HIF-2A TRANSCRIPTION FACTOR

Gaelan Melanson, and Jim Uniacke.University of Guelph.

Oxidative stress is a result of a cellular imbalance between the production and elimination of reactive oxygen species (ROS). The hypoxia-inducible factor (HIF) 2? transcription factor, encoded by the EPAS1 gene, has been implicated in protecting cells against oxidative stress. EPAS1-/- mice exhibit multiple metabolic pathologies and increased ROS production. Furthermore, HIF-2? has been shown to target the expression of many anti-oxidant enzymes such as manganese superoxide dismutase and frataxin. Interestingly, intermittent hypoxia has been implicated in oxidative stress through enhanced HIF-2? degradation via calpain proteases. Thus, further investigation of HIF-2? attenuation will provide better insight into how hypoxia and HIF-2? dysregulation contribute to oxidative stress. Recently, the ubiquitin-like modifier interferon-stimulated gene 15 (ISG15) has been shown to down-regulate activity of the HIF-2? homolog, HIF-1?, in hypoxia. However, there is no information regarding the effects of ISGylation on HIF-2?. Using the model cell line HCT116, our data suggests ISG15 is a suppressor of HIF-2? transcriptional activity. Overexpression of the ISGylation system enhanced HIF-2? degradation in hypoxia. Furthermore, qPCR analysis revealed that HIF-2? target transcripts significantly decreased in the presence of ISGylation in hypoxia. Interestingly, the ISGylation system has been identified to contain HREs. qPCR analysis suggests that ISGylation expression is only transiently induced in hypoxia. Therefore, ISGylation likely participates in a negative feedback loop of HIF-2?-mediated gene expression in hypoxia. This work highlights ISG15 as a regulatory modifier of HIF-2? transcriptional activity, and provides insight into a potentially new avenue for oxidative stress management.

PPA16

OPTIMIZATION AND VALIDATION OF IRDYE800CW FOR THE DIRECT DETECTION OF PROTEIN OXIDATION IN MUSCLE LYSATE USING IN-GEL AND MICROPLATE ASSAYS

Ali Nejatbakhsh[1], Christopher G. R. Perry[1]; Patrick C. Turnbull, and Aimee Landar[2]. [1]YorkUniversity; [2]Department of Pathology, University of Alabama at Birmingham, Alabama, USA.

Direct detection of cysteine redox state in mixed tissue lysates with maleimide-based fluorophores is growing in popularity with a variety of probes available or emerging on the market. However, successful application of this method using in-gel and in-well assays can be highly dependent on various assay conditions and requires extensive optimization and validation for each specific flourophore. IRdye800CW Maleimide is purported to be a highly sensitive maleimide-based infrared fluorophore specific to reduced thiols. It has been reported to detect the redox state of immunoprecipitated proteins, but has yet to be validated as a novel tool to detect redox conditions throughout the proteome in lysate where multiple experimental conditions might interfere with its efficacy using common and cost-effective in-gel and in-well assays. In this study we tested the efficacy of IRDye800CW Maleimide in detecting protein redox state in cardiac muscle using mini-gel SDS-PAGE and an in-well microplate approach. We found that filtering lysates through a desalting column (<7kDa) prior to dye labelling was essential for detecting cysteine labeling. This was partially explained by a small removal of cysteine-rich glutathione (GSH) but may be due to removal of other small thiols. Likewise, the presence of CHAPS detergent interfered with labeling as shown by limited effectiveness of in vitro

treatment with reductant (TCEP) or oxidant (H2O2 + NaI) controls. In Tris buffer without CHAPS, H2O2 with NaI decreased signal intensity which was rescued by subsequent reduction with TCEP in both in-gel (25-100 kDa) and in-well assays. These results were matched by similar changes in GSSG and GSH/GSSG measured by UV/fluorescent HPLC. Further validation with heat stress –an inducer of oxidative stress - decreased dye fluorescence with matching shifts in GSH/GSSG at both 40^0C and 49^0C. Changes in dye fluorescence were also related to similar shifts in protein carbonylation at 49^0C but not 40^0C. IRdye800CW is effective at capturing changes in cysteine redox state throughout the proteome using simple mini-gel and microplate approaches common to many laboratories but only in the absence of buffer detergents and small molecular weight interfering compounds.

PPA17

EFFECT OF SECOISOLARICIRESINOL DIGLUCOSIDE ON ANTIOXIDANT STATUS AND REDOX SIGNALING IN CARDIAC IRON OVERLOAD

Ashley Nemec-Bakk[1], Stefanie Kirk[1], Stephanie Puukila[2], Simon Lees[3], and Neelam Khaper[3]. [1]Department of Biology, Lakehead University; Biotechnology, Lakehead University[2]; Biology/NOSM[3].

Iron is essential to many biological and metabolic processes used in all organisms, but excess iron can result in complications such as cirrhosis, diabetes, and heart failure. Cardiac iron overload has been linked to increased oxidative stress and cell death. Oxidative stress has been shown to play a role in cardiovascular diseases. Antioxidants have been shown to counter the effect of oxidative stress by scavenging reactive oxygen species. Protein kinase B (Akt) promotes cell survival by regulating growth, antioxidant production, and cell death in cardiomyoctes. The aim of this study was to examine the antioxidant effect of Secoisolariciresinol diglucoside (SDG), a phytochemical extracted from flax seeds, in cardiac iron overload condition. We have previously demonstrated that SDG attenuated the increase of inflammatory and apoptosis mediators caused by iron treatment. H9c2 cardiomyocytes were incubated with 50μM iron for either 6 or 24 hours and/or received a SDG pretreatment of 250μM for 24 hours. Western blot was used to determine the expression of redox sensitive proteins in each treatment group. Pretreatment of SDG resulted in increased protein expression of the antioxidant catalase after 24 hours of iron treatment and decreased Akt activity after 6 hours of iron treatment. Iron treatment also increased necrosis after 6 hours of iron treatment. Future studies are directed towards examining the role of AMPK signaling in this model.

PPA18

COMPARISON OF DIFFERENT POLYPHENOLS ON INHIBITION OF BENZO[A]PYRENE CARCINOGENESIS: INFLUENCES ON OXIDATIVE STRESS AND MITOCHONDRIAL BIOGENESIS

Kosar Omidian, Hossein Rafiei and Brian Bandy.University of Saskatchewan.

Benzo[a]pyrene (BaP) is a potent polycyclic aromatic hydrocarbons (PAHs) carcinogen which produces DNA adducts in both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). We hypothesized that BaP causes mutations which decrease mitochondrial function and increase generation of reactive oxygen species (ROS), and subsequently inflammation, and that dietary polyphenols can protect against this action by stimulating mitochondrial biogenesis and decreasing mitochondrial ROS. In an in vitro model of carcinogenesis, Bhas 42 mouse fibroblast cells were pre-treated with 5 μM polyphenol (quercetin, berberine, catechin, resveratrol, or

cyanidin) for 2 hours then treated with 4 µM BaP for 12 and 24 hours. We measured total intracellular ROS, mitochondrial superoxide (MitoSOX red staining), mRNA expression of mitochondrial uncoupling protein 2 (UCP2) (biomarker of oxidative stress and tumorigenesis), tumor necrosis factor alpha (TNF-α) (biomarker of inflammation), SIRT1, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), and citrate synthase (biomarkers of mitochondrial biogenesis), nDNA and mtDNA-encoded respiratory complex subunits (biomarkers of mitochondrial dysfunction), and transformation assay (to determine neoplasia). BaP significantly increased intracellular ROS (57%), mitochondrial superoxide (174%), and UCP2 (70%) compared to untreated cells. Almost all of the polyphenols prevented or strongly inhibited these increases, with cyanidin most strongly inhibiting mitochondrial superoxide generation. BaP significantly decreased mRNA expression of nDNA-encoded (NDUFS8, 30%; ATP5a, 35%) and mtDNA-encoded (MTCYB, 28%; MTCO1, 24%) mitochondrial complex subunits, and SIRT1 (37%) but non-significantly PGC-1α and citrate synthase. The presence of one or more polyphenols reversed these changes, and in many cases increased the expression of mRNA for mitochondrial proteins and mitochondrial biogenesis factors to levels significantly higher than untreated cells. BaP increased expression of TNF-α by more than 3-fold and all polyphenols strongly inhibited this effect. Finally, BaP increased the number of foci by more than 5-fold and most of polyphenols inhibited this effect, with resveratrol having the strongest inhibition.Together the data indicate that polyphenols have potential to inhibit BaP-induced increases in mitochondrial superoxide, intracellular ROS, inflammatory cytokine production and foci number, and to prevent decreases in expression of mitochondrial protein genes involved in mitochondrial function and biogenesis.

PPA19

NEURONAL AGING AND OXIDATION: DOES OXIDATIVE STRESS INDUCE MICRO-ARCHITECTURAL CHANGES IN LIVING MEMBRANES?

Alexander C. Perry, Petra M. Hermann and Willem C. Wildering.University of Calgary.

Aging is a physiological process common to most complex organisms that, in the case of animals, is typically accompanied by a host of neurobiological changes including progressive memory and learning deficiencies. Yet, the fundamental molecular mechanisms underlying these age-associated changes are still not completely understood. One of the cornerstones of biological aging theories is the concept of oxidative stress: the idea that aging is associated with the accumulation of oxidative damage to the building blocks of life. While most molecules of life, and therefore most of its structures, are vulnerable to oxidation, some are more so than others. One particularly oxidation-prone molecular domain is that of phospholipid membranes, specifically the polyunsaturated fatty acids (PUFAs) they contain.In silico and model membranes, studies predict that PUFA oxidation can induce changes in the microarchitecture of plasma- and organelle- membranes that may alter their barrier functions. This study centers on the idea that these processes are a key factor in the functional decline of the aging nervous system. This theory is supported by previous work in our laboratory that implicates oxidative stress-induced recruitment of phospholipase A2 (PLA2) in behavioural and neurobiological deficiencies in a snail model system of biological aging.We aim to investigate the causal link between the oxidation of the plasma membrane, PLA2 activation, and membrane integrity, with a focus on PUFA oxidation-induced impairment of plasma membrane barrier functions. To examine this, isolated neurons and young and old whole brains have been evaluated for their changes in membrane permeability using the cationic and anionic fluorescent membrane probes propidium iodide (PI) and carboxyfluorescein (CF), respectively. The data indicates a significant increase in PI membrane permeability of old preparations

relative to young preparations under control conditions, and a significant decrease in PI membrane permeability in old preparations under conditions of experimentally induced plasma membrane oxidation. In contrast, the CF data shows no significant differences in probe distribution under either experimental condition. Together, this data indicates that neuronal plasma membrane barrier function indeed declines with age and oxidative stress, but also argues that this decline cannot be easily generalized.

PPA20

CAN DIETARY POLYPHENOLS INHIBIT NON-ALCOHOLIC FATTY LIVER DISEASE? PROTECTIVE ROLES AGAINST FIRST AND SECOND HITS INVOLVED IN THE PATHOGENESIS OF THE DISEASE

Hossein Rafiei, Brian Bandy,and Kosar Omidian.University of Saskatchewan, College of Pharmacy and Nutrition.

Nonalcoholic fatty liver disease (NAFLD) affects 24% of the general population and can progress to nonalcoholic steatohepatitis (NASH) and cirrhosis. Hepatic lipid accumulation (steatosis), reactive oxygen species (ROS), inflammatory cytokines, and mitochondrial dysfunction have been identified as important contributors to NAFLD and progression to NASH. Dietary polyphenols have previously been found to help protect against hepatic steatosis and mitochondrial dysfunction in NAFLD, but the most effective and mechanisms involved are not well known. In an in vitro model of NAFLD using cultured human HepG2 hepatocytes exposed to free fatty acids, we studied the effects of five dietary polyphenols (catechin, resveratrol, quercetin, berberine, cyanidin) on hepatic steatosis, oxidative stress, and mitochondrial biogenesis and function.Cells were pre-treated with 10 µM polyphenols for 2h, exposed to 1.5 mM oleic acid for 24h, and then evaluated for intracellular fat accumulation (Nile Red staining), ROS, mitochondrial content (MitoTracker Green), mitochondrial membrane potential and relative mRNA expression (using RT-qPCR) of genes involved in steatosis, oxidative stress and inflammation, including carnitine palmitoyltransferase1 (CPT1A1), peroxisome proliferator-activated receptors (PPARα, PPARγ), fatty acid synthase (FAS), mitochondrial uncoupling protein 2 (UCP2), mitochondrial superoxide dismutase (Mn-SOD), and tumor necrosis factor (TNFα). Oleic acid significantly induced intracellular steatosis (150%) and ROS generation (127%), and decreased mitochondrial content and membrane potential. All of the polyphenols significantly decreased intracellular lipid accumulation (42-58%) and ROS generation (42-77%), and some polyphenols prevented loss of mitochondrial content and membrane potential. Oleic acid decreased the expression of UCP2, consistent with the increased ROS, while catechin, quercetin and cyanidin prevented this decrease. Oleic acid increased the expression of TNFα and lipogenic FAS while some polyphenols inhibited the increase. Oleic acid increased the expression of CPT1A1 by 200%, suggesting an adaptive increase in mitochondrial fatty acid β-oxidation, and all polyphenols except cyanidin further increased the expression to even more than oleic acid alone. Together the data show that dietary polyphenols protect against steatosis, ROS, inflammation, and mitochondrial dysfunction through modulating the expression of genes involved in lipogenesis, β-oxidation, oxidative stress, inflammation, and mitochondrial biogenesis and should be studied further as possible dietary treatments for NAFLD.

PPA21

DETERMINING THE EFFECTS OF MICROTUBULE STABILIZING AND DESTABILIZING CHEMOTHERAPY DRUGS ON MITOCHONDRIAL BIOENERGETICS IN SKELETAL MUSCLE

SofhiaRamos, Meghan Hughes, and Dr. Christopher Perry.York University.

Recent functional evidence suggests microtubules play a role in muscle mitochondrial bioenergetics through an interaction between the microtubule subunit tubulin and the voltage depended anion channel (VDAC). Given VDAC regulates mitochondrial ADP import and membrane potential, this model suggests microtubules may regulate mitochondrial oxidant emission by modulating VDAC permeability to ADP. We hypothesize that stabilizing microtubules (taxol) will decrease VDAC permeability to ADP and increase H2O2 emission whereas destabilizing microtubules (vinblastine) will increase VDAC permeability to ADP and amplify the degree by which ADP reduces H2O2 emission. Soleus (Sol) and white gastrocnemius (WG) skeletal muscles were harvested from C57BL/6J mice (n=6). Sol and WG muscles were separated into muscle bundles and permeabilized with saponin (40µg/ml). All muscles were incubated for 2hr in either taxol or vinblastine before measuring ADP-suppression of succinate-induced H2O2 emission with high-resolution spectrofluorimetry. Succinate-induced H2O2 emission (0mM ADP) was increased in WG by taxol (control; 111.0 pmol/min/mg d.w., taxol; 160.0 pmol/min/mg d.w., p=0.001) but was unchanged in Sol with any drug. However, both drugs in WG and Sol enhanced the effect of ADP-suppression of H2O2 emission by 37-57% vs control at a range of ADP concentrations. Hence, in both WG and Sol muscle, the ability of ADP to suppress H2O2 emission was enhanced by both microtubule stabilizers and destabilizers in spite of higher succinate-induced H2O2 emission in the absence of ADP. Subsequent experiments will determine if the enhanced ADP sensitivity on suppressing H2O2 emission is the result of a detrimental collapse of mitochondrial membrane potential in response to excessive VDAC permeability. Specifically, our next phase will measure tubulin-VDAC protein-protein interaction at a 12 angstrom resolution (proximity ligation assay), the associated mitochondrial membrane permeability and the activation of cell death programs in relation to muscle dysfunction. The current findings of greater ADP-mediated suppression of H2O2 emission also support the emerging concept that microtubules are a direct regulator of mitochondrial bioenergetics in skeletal muscle and raise intriguing questions regarding the role of mitochondria in chemotherapy-induced myopathy.

PPA22

THE MANIPULATION OF CYTOSOLIC NUCLEOSIDE DIPHOSPHATE KINASE IN TRANSGENIC POTATO ROOTS AFFECTS RESPIRATORY METABOLISM LEADING TO ALTERATION IN REACTIVE OXYGEN SPECIES LEVELS AND REDOX REGULATION OF STARCH METABOLISM

JeanRivoal, Sonia Dorion, and Audrey Clendenning.Université de Montréal.

Nucleoside diphosphate kinase (NDPK) catalyzes the transfer of the terminal phosphate from a donor nucleoside triphosphate to an acceptor nucleoside diphosphate. In all organisms, NDPKs are considered as housekeeping enzymes involved in energy metabolism and homeostasis of intracellular nucleoside triphosphate pools. Plant NDPKs are found in various compartments, and some isoforms have been implicated in diverse functions including oxidative stress management. In this study, we focus on cytosolic NDPK

(NDPK1), the most abundant plant NDPK isoform. We used a targeted metabolomic approach and measurement of physiological parameters to investigate the effects of the genetic manipulation of NDPK1 expression on respiration, carbon metabolism and reactive oxygen species (ROS) production in potato (Solanum tuberosum) roots. Sense and antisense NDPK1 constructs were introduced in potato using Agrobacterium rhizogenes to generate a population of root clones displaying a 40-fold difference in NDPK activity. Root growth, glycolytic flux and respiration were positively correlated with NDPK1 expression. No significant variation was observed in the adenylates pools among transgenic roots. However, changes in NDPK1 expression levels led to shifts in the pools of important intermediates in carbon metabolism and in root starch content, which was negatively correlated to NDPK1 expression. Genetic manipulations of NDPK1 expression level also led to alterations in the redox state of ADP-glucose pyrophosphorylase (AGPase), a key enzyme in starch synthesis. Furthermore, there was a positive correlation between NDPK1 expression and the levels of ROS in root tissues. These data are consistent with the hypothesis that, in antisense clones, low cytosolic consumption of ATP by NDPK1 leads to low respiration and ROS production. These conditions favor the provision of substrate for reduced (activated) AGPase in the plastid, leading to increased starch levels. In sense clones, high NDPK activity in the cytosolic compartment of sense roots stimulates respiration through ATP consumption. As a consequence, mitochondrial ROS production is increased, resulting in oxidation (inactivation) of AGPase.

PPA23

HUMAN CELLS CULTURED UNDER PHYSIOLOGICAL OXYGEN HAVE LESS ROS-INDUCED DNA DAMAGE AND UTILIZE TWO CAP-BINDING PROTEINS TO RECRUIT DISTINCT MRNAS FOR TRANSLATION

Sara Timpano, and James Uniacke.Department of Molecular and Cellular Biology, University of Guelph.

Translation initiation is a focal point of translational control and requires the binding of eukaryotic initiation factor 4E (eIF4E) to the 5' cap of mRNA. Under conditions of extreme oxygen depletion (hypoxia), human cells repress eIF4E and switch to an alternative cap-dependent translation mediated by a homologue of eIF4E, eIF4E2. This homologue forms a complex with the oxygen-regulated hypoxia inducible factor (HIF) 2α and can escape translation repression. This complex mediates cap-dependent translation under cell culture conditions of 1% oxygen (to mimic tumor microenvironments), while eIF4E mediates cap-dependent translation at 21% oxygen (ambient air). However, emerging evidence suggests that culturing cells in ambient air, or "normoxia", is far from physiological or "normal". In fact, oxygen in human tissues ranges from 1-11% or "physioxia". Here, we show that two distinct modes of cap-dependent translation initiation are active during physioxia, and act on separate pools of mRNAs. The oxygen-dependent activities of eIF4E and eIF4E2 are elucidated by observing their polysome association, and the status of mTORC1 (eIF4E-dependent) or HIF-2α expression (eIF4E2-dependent). We have identified oxygen conditions where eIF4E is the dominant cap-binding protein (21% normoxia or standard cell culture conditions), where eIF4E2 is the dominant cap-binding protein (1% hypoxia or ischemic diseases and cancerous tumors), and where both cap-binding proteins act simultaneously to initiate the translation of distinct mRNAs (1-11% physioxia or during development and stem cell differentiation). These data suggest that the physioxic proteome is generated by initiating translation of mRNAs via two distinct, but complementary, cap-binding proteins

PPA24

HUMAN CELLS CULTURED UNDER PHYSIOLOGICAL OXYGEN CONDITIONS HAVE HIGHER VIABILITY AND LOWER REACTIVE OXYGEN SPECIES

Brianna Guild, Erin Specker, and James Uniacke, Department of Molecular and Cellular Biology, University of Guelph

Human cell culture is widely used as a model to investigate and characterize molecular and biochemical processes. Oxygen availability, a crucial variable in human physiology, is often neglected in cell culture. Indeed, cell culture is typically performed in ambient air (21% oxygen), termed normoxia, while the documented physiological oxygenation range of human tissues, termed physioxia, is 1–11%. Several studies have suggested beneficial effects of culturing mammalian cells in low oxygen; however, to date there has been no systematic investigation of human cell cultures within the physioxic range. Here we show that culturing human primary glioblastoma cells within physioxia promotes cell viability and metabolic activity, decreased cellular reactive oxygen species levels and optimal mitochondria morphology. Our data suggests that culturing human cells in physioxia may provide a better model for making physiological inferences.

PPA25

NUTRITIONAL TARGETING OF MITOCHONDRIA IN CANCER: LIPID INCUBATION INCREASES H2O2 EMISSION IN HT29 AND MCF7 ADENOCARCINOMAS BUT NOT IN NON-CANCER EPITHELIAL CELLS.

Patrick C. Turnbull, Ali Nejatbakhshand Christopher GR. Perry.Molecular, Cellular and Integrative Physiology School of Kinesiology and Health Sciences, York University.

A benchmark of cancer cells is the reliance on glycolysis characterized by decreased mitochondrial oxidative phosphorylation (the Warburg effect). Recent evidence suggests that fatty acids (palmitoylcarnitine) may force a shift to mitochondrial oxidative phosphorylation in HT29 colon cancer causing cell death, yet non-cancer cells appear to be resistant. These results present the intriguing possibility that 'lipid-therapy' might be well tolerated by healthy cells while retaining potency in cancer. However, the mechanism of lipid-induced cancer cell death is not known. Our pilot investigation hypothesized that cancer cells will produce elevated levels of H2O2, the major oxidant emitted by mitochondria, in association with increased reducing equivalents (ie. NADH) from beta-oxidation while non-cancerous cells will be resistant to this fat exposure despite enhanced NADH generation. Two adenocarcinomas (HT29 colon and MCF7 breast cancer) as well as a non-cancer colonocyte CCD-841 were incubated for 3 hours with varying concentrations of intralipid (0.625%-2.5%, or ~20-90mM) containing emulsified polyunsaturated fatty acids. Using in-well spectrofluorometry, we measured H2O2 emission in living adherent cells (Amplex Ultrared) and assessed cell viability through NADH production using a tetrazolium salt (XTT). CCD-841 and HT29 increased NADH production when exposed to increasing amounts of fat with no change in MCF7 after 3 hours of lipid incubation. However, only the cancerous MCF7 and HT29 cells demonstrated elevated H2O2 emission when incubated with increasing amounts of fat while no change was observed in the non-cancerous CCD-841. These results suggest the increased H2O2 emission in HT29 cells followed increased NADH generation from fat oxidation, whereas non-cancer CCD-841 cells tolerated increased NADH without increasing H2O2 emission. In MCF7, the increased H2O2 emission despite no change in NADH at 3 hours might suggest NADH peaked at an earlier time point, which might suggest the existence of heterogeneous temporal coupling

between the rate of fat oxidation and oxidant production across cancers. Using the present findings of heightened oxidant emission in response to lipids in cancer, our next experiments will elucidate the dose-time responses between lipid-activation of oxidative phosphorylation and oxidant emission in relation to cellular redox environment and induction of cell death.

PPA26

ROLE OF VITAMIN C IN TET-MEDIATED OXIDATION OF 5-METHYLCYTOSINE TO 5-HYDROXYMETHYLCYTOSINE IN THE BRAIN OF YOUNG MICE

J. Richard Wagner[1,2],Carolyne Marie Lowry[3], Rona Graham[3], and Michel Lebel[3]. [1]Department of Nuclear Medicine and Radiobiology, Université de Sherbrooke; [2]Centre de recherche sur le vieillissement, Université de Sherbrooke; [3]Centre de recherche du CHU de Québec, Université Laval.

5-Hydroxymethylcytosine (5hmC) is a recently discovered DNA epigenetic mark. The level of 5hmC is several fold higher in the brain compared to other tissues in rodents and humans (0.5-1.0% of total cytosine in genomic DNA). Low levels of 5hmC have been associated with cancer, in particular severe grade brain gliomas, as well as serious neurological disorders. 5hmC arises from the enzymatic oxidation of 5mC by TET 1-3 enzymes, a family of alpha–ketoglutarate and Fe(II)-dependent dioxygenases. Vitamin C is a cofactor of TET activity such that it reduces Fe(III) allowing for efficient recycling of enzyme activity. We hypothesized that low vitamin C inhibits TET activity and decreases the level of 5hmC in the brain. This hypothesis was investigated using Gulo -/- knock-out mice that do not synthesize vitamin C. The mice were supplemented with vitamin C in their drinking water for 4 months with 0.01% followed by 0% (v/v) in the last month (group 1); with 0.01% for the entire 4 months (group 2), and finally with 0.4% throughout the 4 months (group 3). The concentration of vitamin C in plasma was non-detectable in group 1 (extreme deficiency), 10 µM in group 2 (similar to the levels in scurvy) and 70 µM in group 3 (normal). The levels of 5hmC in genomic DNA of tissue were determined by DNA extraction and digestion to its component 2'-deoxyribonucleosides followed by LC-MS/MS analysis. The results indicate that the amount of 5hmC increased by about 25% in the cerebellum in going from group 1 with extremely low levels of vitamin C to group 3 with normal levels of vitamin C. Likewise, vitamin C increased the levels of 5hmC in the cortex, whereas changes in striatum and hippocampus regions were minor or not significant. Thus, low levels of vitamin C may contribute to a decrease in 5hmC in the brain although the levels remain high even in the absence of vitamin C. These results suggest that other reducing agents may substitute for vitamin C in order to maintain TET activity and high levels of 5hmC in the brain.