

CAANCB Student Oral Presentation Abstracts

C1 NECROSTATIN-1 PROTECTS HYPOXIA INDUCED NEURONAL DEATH BY INHIBITING BNIP3.

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Necrostatin-1 (Nec-1) has been shown to be highly neuroprotective in ischemic neuronal death in vivo and in glutamate-induced cell death in vitro. Previously we reported that the mitochondrial death-inducing protein BNIP3 was upregulated in hypoxia and stroke, and caused neuronal cell death in a caspase-independent manner. The BNIP3-induced cell death pathway involves the interaction of dimerized BNIP3 with mitochondria to cause mitochondrial dysfunction, and subsequent release and nuclear translocation of the mitochondrial protein endonuclease G (EndoG). Here we show that Nec-1 inhibits BNIP3 function by preventing BNIP3 from interacting with mitochondria. Recombinant BNIP3 protein, when incubated with freshly isolated mitochondria, was able to integrate with mitochondria, possibly through its interaction with the mitochondrial membrane protein VDAC, and induce release of mitochondrial proteins including EndoG. When Nec-1 was added to the incubation, it significantly disassociated the binding of BNIP3 to mitochondria and prevented the release of EndoG. In primary cortical neuronal cultures, hypoxia induced significantly accumulation of BNIP3 expression and cell death. Inhibition of BNIP3 alone by RNAi significantly reduced neuronal cell death by 25%. Treatment with Nec-1 at the concentrations of 25 μ M and 50 μ M showed similar reduction of cell death rates as by the knockdown of BNIP3 in the same hypoxic condition. Nec-1 treatment did not change the levels of BNIP3 expression, but decreased the amount of mitochondria-bound BNIP3 dose-dependently. Our data suggests that Nec-1 could be an inhibitor for the BNIP3 cell death pathway.

C2 EFFECTS OF AGING ON MYELIN OLIGODENDROCYTE GLYCOPROTEIN AND OLIG2 EXPRESSION IN THE ROSTRAL CORPUS CALLOSUM OF MICE.

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Oligodendrocytes(OLs) are a cell type in the CNS that can readily be induced to wrap axons in a smooth fatty protein called myelin. Myelin insulates the axon and is critical for all bodily movements and functions. The myelinating ability of OLs is essential to the proper repair of CNS injury and yet the efficiency of OL remyelination declines with age. This age related impairment of remyelination is relevant to multiple sclerosis (MS), from which patients may suffer for several decades, and to cognitive impairments accompanying several neurological disorders, including AD, PD and schizophrenia. Little is known of the specific role(s) played by the factors that govern the expression of genes needed for OLs to myelinate axons. To study myelination and remyelination during aging, we developed an animal model that uses cuprizone (a copper chelator) in the diet to demyelinate axons in the rostral corpus callosum (RCC) of mice aged 2 to 16 month-old. This animal model has enabled us to characterize the expression profiles of several OL phenotypic markers and transcriptional factors (TFs) during both the demyelination and remyelination phases in the RCC. Our results indicate an age-related decline in the expression of myelin oligodendrocyte glycoprotein (MOG) and Olig2 TF in the RCC of mice brains. In addition, the expression of Olig2 increased after cuprizone treatment and was significant in older mice, although the OL marker, platelet derived growth factor alpha receptor (PDGFalphaR) did not show a significant age-related change after cuprizone treatment. Cell count data analysis revealed that Olig2 cytoplasmic expression was significantly increased in the early staged PDGFR+ve/Olig2+ve OLs in 12 month-old mice after cuprizone recovery.

**C3
INSULIN-LIKE GROWTH FACTOR TYPE-I β
SUBUNIT RECEPTOR EXPRESSION IN CIGARETTE
SMOKE EXTRACT-EXPOSED FETAL RAT LUNG.**

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Smoking during pregnancy exposes the fetus to 4000 different potentially genotoxic agents of cigarette smoke. In the developing embryo and fetus good evidence supports the role of the Insulin-like Growth Factor (IGF) axis in growth regulation. One component of this axis, IGF-Type I receptor (IGF-IR) is a tyrosine kinase receptor and the beta subunit of this receptor is essential in cellular proliferation and survival. The present study is focused on the effects of cigarette smoke extract (CSE) on IGF-IR expression in developing fetal rat lung cells. Cell cultures from two lung cell types, fibroblasts and Type II alveolar cells (AECs) were isolated from Sprague Dawley fetal rats at 21 gestational days. Data were derived from 15-18 pooled litters. Isolated cell types were exposed to CSE at concentrations from 1-20% for 24 hours. IGF-IR expression was determined by western blotting on 7% polyacrylamide gels. Subsequently, transfer blots were blocked in 5% skim milk before being exposed to IGF-IR beta rabbit polyclonal antibody at 1:1000 dilution. Antibodies to beta actin were used as loading controls. Microscopic imaging was done to assess CSE induced morphological changes. Cell proliferation and cell viability studies were done concurrently. Results indicate that IGF-IR is expressed in both cell types. IGF-IR expression profile is in accordance with the different concentrations of CSE, with no significant change in expression at 1-5%. However, IGF-IR expression was reduced at 10% CSE and above. In addition CSE concentrations above 10% for 24hours of exposure significantly reduced cellular proliferation and viability in a dose dependent manner. The level of IGF-IR expression by developing fetal rat lung cell types suggests its importance during cellular replication and differentiation. The cellular exposure to cigarette smoke extract differentially affects fetal lung cell viability, proliferation and expression of a major regulator of growth, IGF-IR. In humans, exposure to primary or secondary smoke products *in utero* may similarly adversely alter fetal lung cell growth and development.

**C4
PROEPIDERMAL GROWTH FACTOR
CYTOPLASMIC DOMAIN AND PROTEASOMAL
DEGRADATION: A NOVEL WAY TO REGULATE
GROWTH IN HUMAN THYROID CARCINOMA.**

Aleksandra Glogowska, Thomas Klonisch, Department of Human Anatomy and Cell Science, Faculty of Medicine, University of Manitoba. **Source of Research Funds:** Deutsche Krebshilfe & the Manitoba Health Research Council (MHRC)

Introduction: Epidermal Growth Factor (EGF)-like growth factors have essential functions during cell proliferation, cell migration, tissue differentiation, and apoptosis. Initially synthesised as transmembrane proform consisting of an extracellular, trans-membrane, and cytoplasmic domain, soluble EGF is released from the extracellular part of proEGF by proteolytic cleavage and binds to membrane-anchored tyrosine receptor kinases of the ErbB1-4 family. Over-expression or hyper-activation of ErbBs correlate with enhanced *in vitro* cell migration and *in vivo* invasiveness of tumour cells. The cytoplasmic domains of EGF-like precursors, including proEGFcytoplasmic (proEGFcyt) domain have unique functions. We have studied the role of proEGFcyt in thyroid cancer. By generating stable transfectants expressing membrane-anchored and cytosolic proEGFcyt, we have unravelled a novel mechanism of proliferation control in human thyroid carcinoma cells.

Methods: PCR cloning and expression of proEGF constructs in human thyroid carcinoma cells(FTC133), Westernblot analysis, BrdU proliferation assay, Immunoprecipitation, proteasome inhibitor assay, MTT assay, cell culture

Results: We have studied the role of the cytoplasmic domain of human EGF (proEGFcyt) and a novel splice version lacking exon 23 (proEGFdel23) in stable transfectants of the human thyroid carcinoma cell line FTC-133. Over-expression of proEGFcyt, but not proEGFdel23 or mock controls, attenuates growth of human carcinoma cells. Exogenous EGF antagonizes this growth-suppressive action of proEGFcyt and causes a significant increase in tumour cell proliferation by EGF-mediated binding to and activation of the ErbB1 as determined by the specific ErbB1 inhibitor AG1478. ProEGFcyt transfectants display significant reduced presence of ErbB1 and ErbB2/neu. We show that the amount of EGFR protein in proEGFcyt transfectants can be significantly increased in the presence of proteasome inhibitors MG132 and lactacystine indicating proteasomal degradation of ErbB1 and 2. Both EGF receptors were hyper-ubiquitinated as a result of a significant down-regulation of ubiquitin-C-terminal hydrolase L1 (UCH-L1) which is important for protein de-ubiquitinylation. This coincided with a markedly higher level of ubiquitinylation of specific proteins in proEGFcyt transfectants.

Conclusions: Here we describe a novel proEGFcyt-mediates molecular mechanism which facilitates proteasomal degradation of ErbB1/2 and down-regulates proliferation of

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thyroid cancer cells. These results may also be important for the regulation of proliferation of other proEGF-expressing cancer cells.

C5

IDENTIFICATION AND CHARACTERIZATION OF BRN3B AS DLX HOMEBOX GENE TRANSCRIPTIONAL TARGETS IN RETINAL DEVELOPMENT.

Qi Zhang, David Eisenstat, Department of Human Anatomy and Cell Science Faculty of Medicine, University of Manitoba. **Source of Research Funds:** Deutsche Krebshilfe & the Manitoba Health Research Council (MHRC).

Introduction: The Dlx1/Dlx2 double knockout mouse has reduced numbers (33% fewer) of late-born retinal ganglion cells (RGC) compared to wild-type littermate controls. Many of these RGC are lost by apoptosis. The Brn3b homeobox gene is important for RGC differentiation and the Brn3b knockout mouse has significant loss of RGC numbers (70% fewer). We hypothesize that Brn3b are direct Dlx downstream transcriptional target.

Methods: Embryonic retinas were dissected and chromatin immunoprecipitation (ChIP) of this tissue was utilized to identify DLX proteins bound to specific DNA regulatory elements of Brn3b. Electromobility shift assays (EMSA) were used to confirm specificity of binding of DLX proteins to these Brn regulatory sequences. Luciferase reporter gene assays were performed to confirm functional effects of DLX binding on Brn transcription in vitro. We generated a Dlx1/Dlx2/Brn3b triple knockout by breeding Brn3b null mice with Dlx1/Dlx2 heterozygotes.

Results: PCR performed after ChIP showed that both DLX1 and DLX2 proteins bound to Brn3b. Using EMSA, recombinant DLX1 and DLX2 bound to Brn3b and specific supershifted bands resulted from the addition of specific DLX1 or DLX2 antibodies. Both DLX1 and DLX2 binding to the Brn3b promoter activated transcription of a luciferase reporter gene in vitro. In the Dlx1/Dlx2/Brn3b triple null retinas, there are more than 90% RGCs loss, and a cell fate switches from RGCs to dislocated amacrine cells.

Conclusion: Brn3b is transcriptionally regulated by both DLX1 and DLX2. The combinatorial knockout mouse missing Dlx1, Dlx2 and Brn3b has a more severe retinal developmental phenotype than either mouse model alone.

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O1 EPIDERMAL GROWTH FACTOR CYTOPLASMIC DOMAIN IS A MAJOR FUNCTIONAL COMPONENT OF THE MEMBRANE-ANCHORING REGION OF PROEGF AND A NOVEL REGULATOR OF ELASTIN INVASIVENESS OF HUMAN THYROID CARCINOMA CELLS.

Aleksandra Glogowska¹, Ekkehard Weber², Cuong-Vu³, and Thomas Klonisch¹, ¹Department of Human Anatomy and Cell Science, Faculty of Medicine, University of Manitoba, ²Department Physiological Chemistry, ³Clinics of Surgery, Martin Luther University, Halle, Germany.

All Epidermal Growth Factor-like molecules are synthesized as membrane-anchored proforms. We and others have shown that the cytoplasmic domains of EGF-like ligands have important biological functions. Employing stable transfectants of the human follicular thyroid carcinoma cell line FTC133 expressing membrane-anchored (FTC133-proEGFctF) and soluble cytoplasmic domain of proEGF (FTC133-proEGFcyt) we show that proEGFcyt is the essential functional element responsible for the transcriptional up-regulation of the lysosomal hydrolases cathepsin- (cath-) B and -D. ProEGFcyt also affects the processing of cath-L. Cath-L has strong elastinolytic activity and was the only cathepsin to be secreted by all FTC133 transfectants. Of all components of the membrane-anchoring region, we identified proEGFcyt to be sufficient in significantly impairing the ability of FTC133 to migrate through elastin matrices when compared with FTC133 stably transfected with the empty plasmid and a natural proEGFsplice mutant construct. Decreased migration through elastin matrix resulted from decreased secretion of cath-L in the FTC133 clones. A similar reduction in elastinolytic activity was observed when FTC133 control or proEGFsplice form transfectants were incubated with a specific cath-L inhibitor suggesting that this elastinolytic activity detected was largely mediated by cath-L. Down-regulation of cath-L in FTC133proEGFctF and -proEGFcyt involved the upregulation of the t-SNARE component SNAP25 as determined by siRNA knock-down of SNAP25 mRNA. Incubation of FTC133-proEGFcyt with soluble EGF reversed this effects and this antagonistic EGF action was mediated by the EGFR. In summary, we provide first evidence for the suppressive role of proEGFcyt on the ability of thyroid carcinoma cell to invade elastin matrices and identify novel opposing modular functions of soluble EGF and the cytoplasmic domain of human proEGF.

O2 IDENTIFYING MOLECULAR TARGETS OF OXIDATIVE DNA DAMAGE.

Yali Xie, Sabine Mai, Jiuyong Xie, and Robert P. Shiu, Department of Physiology, Manitoba Institute of Cell Biology, University of Manitoba. **Source of Research Funds:** The Thorlakson Foundation.

Oxidative DNA damage has been considered a key factor in aging and age related diseases such as cancer, but the underlying molecular mechanisms are not clear. We have generated knockout mice and cell lines deficient in oxidative DNA damage repair enzymes OGG1 and MYH which remove the most frequent mutagenic base lesion 7,8-dihydro-8-oxoguanine (GO) and the resulting mismatched adenine to prevent G to T mutations. Ogg1 and Myh double knockouts predispose mice to various tumors. In lung tumors, k-ras, but not p53, is frequently targeted by oxidative DNA damage, suggesting specific gene-targeting of oxidative stress in a tissue. In other tumors, no k-ras mutations were found, suggesting the existence of different targets. Therefore, we examined telomeres, as they contain G-rich (TTAGGG)_n tandem repeats and whose dysfunction causes genomic instability, accelerating tumorigenesis and aging. We found that telomere aggregations were significantly increased in non-proliferating tissue liver and proliferating tissue intestine of the mice. Telomeres were also significantly shortened in intestine cells. Moreover, exogenous oxidants also increased multinucleation and centrosome amplification in the Myh^{-/-}Ogg1^{-/-} cell line, which are common in cancer cells. Thus, our study suggests that oxidative stress-accelerated aging is likely through increasing telomere shortening. Moreover, k-ras, telomeres and centrosomes are likely downstream targets of oxidative stress in Myh^{-/-}Ogg1^{-/-} background, suggesting multiple pathways in tumorigenesis caused by oxidative stress.

O3 TGFβ-MEDIATED FIBROSIS PREDOMINATES FOLLOWING MUSCLE CONTUSION IN OLD RATS.

Melanie Wilcox, Ahmed Ghaly and Daniel Marsh, Department of Anatomy and Neurobiology, Dalhousie University. **Source of Research Funds:** NSERC.

After contusion, muscles of old rats have limited regenerative capacity. We hypothesized that oxidative stress, inherent in aged tissue, may modulate the inflammatory response to injury and induce a TGFβ-mediated fibrotic response. We further hypothesized that this fibrotic response compromises myogenic regeneration of the injured muscle. We compared uninjured and contused tibialis anterior muscles of 2, 16 and 24 month-old rats, harvested after 8 h, 3 days and 21 days. Oxidative stress in the muscle was measured by determining the ratio of glutathione in reduced

(GSH) and oxidized (GSSG) forms, by Western blots for GP91^{phox} levels, and by lipid peroxidation assay. All measurements indicated elevated levels of oxidative stress in uninjured muscle of old compared to young rats. After contusion, oxidative stress was increased significantly in muscle of old and adult, compared to young rats. Similarly, markers of inflammation (myeloperoxidase and CD68 content) were significantly greater in contused muscles of adult and old, compared to young rats. Gene expression of markers from the fibrotic (smad3, TGFβ1, collagen1A2), myogenic (myogenin, myosin light chain, Spp-1), and adipogenic (PPAR-γ2) pathways were measured by RT-PCR. Protein levels were measured by Western blot or by ELISA. In contused muscle, mRNA transcripts and protein levels of fibrotic markers, such as smad3 and TGFβ1, were significantly higher in muscle old rats than young. After injury, mRNA transcript levels and protein content of myosin light chain showed higher expression in young muscle, but reduced expression in old muscle. Levels of PPAR-γ2 mRNA transcripts suggested an increase in adipogenesis in old muscle. Following contusion, levels of mRNA transcripts indicated normal regeneration in young muscle, whereas, in old muscle there was an increased fibrotic response. Our data suggests that increased levels of oxidative stress in muscle of old rats exacerbates TGFβ-mediated fibrosis and inhibits myogenesis.

**O4
AGE-RELATED CHANGES IN MONOCYTE AND LYMPHOCYTE CYTOKINE PRODUCTION AND THEIR MODULATION FOLLOWING AEROBIC EXERCISE.**

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It is known that multiple physiological changes accompany aging, prominent amongst these being an alteration in immune functioning. Both numbers of responding cells, and more particularly their biological function have been reported to be altered by numerous investigators. Particular attention has been paid to evidence for an increased inflammatory state in aged individuals, believed in part to be associated with decreased anti-oxidant activity with age, and decreased T lymphocyte reactivity (important for protection from viral infection, and a component of regulation of autoimmune disease and cancer). To date few studies have documented interventions able to produce a consistent change in any specific age-related immune alterations. We have investigated inflammatory cytokine production (IFN and TNF) from in vitro stimulated monocytes or lymphocytes respectively isolated from PBL of healthy normal (age<30yr) or aged (>60yr) volunteers (8 subjects (Ss) and 21Ss

respectively), and the changes which occur in these parameters following a 3 month course of supervised aerobic exercise (8Ss and 18Ss respectively). Monocytes were stimulated with poly I:C, and lymphocytes with PHA. Cytokines were measured in culture supernatants at 30hr, using commercial ELISA kits. In addition, FACS analysis was performed to assess concomitant changes in cell subsets. Significant increases in lymphocyte TNF production, and decreased monocyte-derived IFN production were seen in stimulated cultures from aged subjects. These were not explained by consistent changes in cell subsets, though there was a general trend towards decreased CD4+ T cell counts with age, and decreased NK cell numbers (and function). Interestingly, exercise produced a modest, though to date not yet significant attenuation of these changes in aged subjects, with no clear differences in healthy young controls. We speculate both that the altered cytokine profile observed, and its attenuation by adherence to an aerobic exercise regime in aged individuals, offers a promising approach both to monitor an important age-related immune variable, and intervene in a manner which may produce a clinically important change. Current approaches are designed to explore a correlation between the exercise-induced changes and susceptibility to community acquire influenza infection in a further targeted study group.

**O5
INTERACTION OF CONNEXIN36 WITH MULTI-PDZ-DOMAIN PROTEIN 1, ZONULA OCCLUDENS-2 AND ZONULA OCCLUDENS-3.**

Xinbo Li* and James I Nagy*, Department of Physiology, University of Manitoba. **Source of Research Funds:** CIHR.

The gap junction protein connexin36 (Cx36) is widely expressed in neurons and was previously shown to interact with the PDZ domain-containing protein zonula occludens-1 (ZO-1). We investigated whether Cx36 is also able to interact with other PDZ domain containing proteins, including multi-PDZ-domain protein 1 (MUPP1) and members of zonula occludens family of proteins zonula occludens-2 (ZO-2) and zonula occludens-3 (ZO-3). In lysates of mouse brain, MUPP1 was shown to co-immunoprecipitate with Cx36, Cx43 and Cx47, but not with Cx30 or Cx32. ATC-3 cells and HeLa cells transfected with Cx36 were found to express ZO-2 and ZO-3, both of which were co-localized with Cx36 at gap junctional cell-cell contacts. In lysates of Cx36-transfected HeLa cells, both ZO-2 and ZO-3 were shown to co-immunoprecipitate with Cx36, whereas Cx36/ZO-2 association was absent in cells transfected with truncated Cx36 lacking its c-terminus SAYV PDZ interaction motif. In vitro pull-down assays revealed that Cx36 interacts with the PDZ10 domain of MUPP1, or PDZ1 domain of either ZO-2 or ZO-3. Truncated Cx36 lacking its PDZ binding motif failed to bind PDZ10 of MUPP1 or PDZ1 domain of either ZO-2 or ZO-3. A fourteen amino acid peptide corresponding

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to the c-terminus of Cx36 inhibited the association of Cx36 with the first PDZ domain of both ZO-2 and ZO-3. These results indicate that Cx36 associates with the PDZ10 of MUPP1 and the first PDZ domain of ZO-2 and ZO-3 and that this association requires the c-terminus SAYV sequence in Cx36. These findings, together with the known association of ZO-2 with a variety of proteins including transcription factors and the known association of MUPP1 with various neurotransmitter receptors and signaling molecules, suggest that MUPP1 and ZO-2 may serve to anchor regulatory proteins at gap junctions composed of Cx36 and MUPP1 may play a linker role for the coexistence of chemical synapses and electrical synapses within the same coupled neurons. Research funded by Canadian Institute for Health Research to JN and XL.

O6

IMAGING OF A PREFRONTAL NEURO-COMPENSATORY EFFECT IN EARLY ALZHEIMER'S DISEASE

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The objective of this research is to investigate possible neurocompensatory responses associated with the early stages of Alzheimer's disease (AD), with the application of high-field functional magnetic resonance imaging (fMRI). We examined changes of the fMRI activation at resting-state as well as at cognitive tasks in mild AD patients and in cognitively intact older adults. The dorsolateral prefrontal cortex (DLPFC) was chosen as a region of interest (ROI) because of its role in executive functions and working memory. Four mild AD patients and twelve age-matched healthy control subjects were scanned on a 4-Tesla Varian-Oxford human imaging system. During the resting state, subjects were instructed to remain relaxed while focusing their eyes on a central fixation for over 60 seconds. Cognitive tasks involved encoding and episodic memory retrieval; all of which involved viewing pairs of standard line-drawn pictures. Functional images were acquired using two-shot spiral readout; 22 axial slices of 5.5 ± 0.5 mm. Resting-state data were processed using independent component analysis and artifacts were filtered. Whole brain activation maps during each cognitive task and ROI analysis in the DLPFC were obtained applying a canonical haemodynamic response function to fit task onset and duration ($p < 0.001$, uncorrected, extent=6). During rest, three components were identified across subjects, with each being characterized as a low frequency response (< 0.1 Hz). Within the DLPFC, both the mean signal change and the statistics in the contrast maps were greater in AD patients than in cognitively healthy older subjects. The cognitive tasks evoked distributed fMRI

activation both in AD and control subjects. While AD patients showed increased DLPFC ROI activation for encoding, in the retrieval task there was no further increase of fMRI activation in DLPFC ROI. In contrast, in healthy subjects, only moderate bilateral DLPFC activation was observed during the encoding tasks, whereas a markedly increased fMRI activation was associated with performance of the recognition task. Our preliminary data suggest that compared to cognitive healthy older adults, patients with mild AD showed increased resting-state and encoding task-induced fMRI activation, probably reflecting an early neurocompensatory response to AD.

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O7

SULFORAPHANE LOWERS SYSTOLIC BLOOD PRESSURE AND PREVENTS RENAL VASCULAR REMODELLING IN SHRSP RATS.

Vijitha Senanayake, Ali Banigesh, Lingyun Wu, Paul Lee, and Bernhard Juurlink, Departments of Anatomy & Cell Biology, Pharmacology and Physiology, University of Saskatchewan. **Source of Research Funds:** Saskatchewan Health Research Foundation and CIHR.

We have previously shown that consumption of glucoraphanin in a food matrix reduced oxidative stress and lowered inflammation as well as blood pressure in Spontaneously Hypertensive Stroke Prone rats (SHRsp). These effects of glucoraphanin are likely mediated by the phase 2 protein-inducing activity of the glucoraphanin metabolite sulforaphane (SUL). To test this idea we administered SUL ($10 \mu\text{Moles/Kg}$) to 5-wk old Spontaneously Hypertensive Stroke Prone rats (SHRsp) for 4 months, and measured blood pressure by an external catheter inserted into the carotid artery. Tissues were also collected for glutathione (GSH) and histological analysis. For comparison, age-matched normotensive Sprague Dawley (SD) rats were treated in the same manner. SHRsp control rats had significantly higher Systolic Blood Pressure (SBP) (141.9 ± 3.0) than control SD rats (119.5 ± 5.7) and SUL treatment significantly lowered SHRsp blood pressure to 129.0 ± 2.4 . There was no effect of SUL treatment on SD rat SBP (118.4 ± 1.8). Since kidney is the major organ responsible for hypertension in SHRsp, we then examined the kidneys for GSH content and histopathological changes. Striking differences between different experimental groups were seen in the renal small arteries/arterioles. We measured the lumen to wall ratio (LWR) in these vessels and the thickness of the arterial wall in SHRsp control rats were the highest (LWR: 7.1 ± 2.3) and the SUL treatment reduced the thickness almost by half (LWR: 3.7 ± 0.9), bringing the thickness down to the level of SD controls (2.3 ± 0.4) or SUL

treated SD (2.6 ± 1.3). Renal oxidative stress is known to be associated with the maintenance of hypertension in SHRsp. SUL is a known potent phase 2 enzyme inducer and induction of these enzymes leads to reduced oxidative stress. Indeed, our study demonstrated that SHRsp on control diet had much lower levels of renal GSH than SUL treated SHRsp. We conclude that phase 2 enzyme induction and subsequent reduction in oxidative stress by SUL leads to lowering of blood pressure and prevention of renal vascular remodelling.

O8 EFFECT OF PARITY ON VASCULAR COMPLIANCE AND COLLAGEN CONTENT.

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Epidemiological studies have revealed that repeated pregnancy is associated with increased risk of cardiovascular disease in later life. During pregnancy there is increased arterial compliance. However, we have shown that in the long term, after reproduction activity has ceased, mesenteric arterial/venous compliance is decreased and there is evidence for degradation of elastic tissues. Collagen fibers are the most abundant components of the extracellular matrix in arteries. Collagen degradation is tightly regulated by the zinc-dependent endopeptidases matrix metalloproteinases (MMPs). Under conditions when the balance of collagen synthesis/breakdown changes, the mechanical and functional characteristics of the vessels are altered. We have previously shown that multiparity induces oxidative stress, which leads to peroxynitrite formation and increased vascular tone. We hypothesized that peroxynitrite also decreases MMP levels in vascular tissues, which then increases collagen content. The experiments were done on aortic and mesenteric vascular beds of multiparous (3-5 pregnancies) and age-matched virgin rats. We examined the effect of multiparity on vascular collagen content using Trichrome Masson staining of paraffin imbedded sections. MMP-2 activity in the vascular tissues was examined by gelatin zymography. Our data demonstrate that parity is associated with decreased MMP-2 activity in mesenteric homogenates (Virgin: 225 ± 71 versus Multiparous: 662 ± 121). Preliminary experiments reveal that there is also increased collagen content in mesenteric and aortic sections. These data suggest that parity decreases vascular compliance, and increases tone via mechanisms involving MMP-regulated collagen degradation which is downstream of peroxynitrite signalling pathway.

O9 ADIPOSE-DERIVED STEM CELLS ARE AN EFFECTIVE THERAPY FOR HEART FAILURE.

Ganghong Tian, Lei Wang, Jixian Deng, Bo Xiang, Jian Wang, Marco Gruwel, John Rendell, Miriam Glogowski, Boguslaw Tomanek, Darren Freed, Roxanne Deslauriers, and Rakesh C. Arora, Institute for Biagnostics, NRC, Winnipeg, Canada.

The implantation of non-cardiac tissue (stem cells) to improve heart failure is an exciting yet still evolving therapy. This study was to determine the utility of adipose-derived stem cells (ASCs) to repair and improve cardiac function following myocardial infarction. Myocardial infarction was created in 34 in-bred Lewis rats by occlusion of their left anterior descending (LAD) coronary artery. One week after the LAD occlusion, the animals were randomly divided into two groups. One group was subjected to intramyocardial injection of ASCs and a second group to intramyocardial injection of cell-culture medium (CCM). Magnetic resonance (MR) imaging showed that left ventricular ejection fraction (LVEF) measured at 1 week and 4 weeks after cell transplantation in the ASC-treated hearts was significantly greater than that of the CCM-treated hearts. LV wall thickness and systolic thickening in the infarct region were also greater in the ASC-treated hearts than in the CCM-treated hearts. Moreover, capillary density in the infarct region was significantly higher in the ASC-treated hearts than in the CCM-treated hearts. Heart tissue sections and the engrafted ASCs isolated from the ASC-treated hearts at end of protocol showed that only 0.5% of the implanted ASCs expressed sarcomeric alpha-actinin and alpha-myosin heavy chain. In conclusion, ASCs significantly improved cardiac function of infarct hearts and may be an effective therapy for myocardial infarction and heart failure. However, only a small percentage of the engrafted cells transdifferentiated into a cardiomyocyte phenotype, suggesting an alternative mechanism of action that requires further investigation.

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O10

UNRESOLVED ISSUES IN THE LINK BETWEEN DOCOSAHEXAENOIC ACID AND ALZHEIMER'S DISEASE.

Plourde M*, Fortier M*, Vandal M*, Tremblay-Mercier J*, Freemantle E*, Bégin M*, Pifferi F*, and Cunnane SC, Research Center on Aging, Department of Medicine, and Physiology and Biophysics, Université de Sherbrooke. **Source of Research Funds:** NSERC, Canadian Foundation for Innovation, Canada Research Chairs Secretariat, Department of Medicine of Université de Sherbrooke, and the Research Center on Aging.

Lower consumption of docosahexaenoic acid (DHA) is commonly but not always associated with a higher risk of cognitive decline and diagnosis of Alzheimer's disease (AD). The objective is to review the available data relating DHA to AD, with emphasis on DHA content of plasma and brain. Our assessment of this literature is that low DHA is not consistently observed in AD plasma or brain. However, in dietary and population studies unrelated to AD, low DHA intake is usually associated with lower plasma DHA. Therefore, at present, there is no clear explanation of why the usual low DHA intake – low plasma DHA relationship appears not to exist in AD. Adding to the confusion, preliminary and inconclusive reports tentatively suggest that dietary DHA could potentially reduce cognitive deterioration in AD. Raising DHA intake by 5-10 fold is well known to raise DHA in plasma phospholipids but, in our experience, does not change free plasma DHA, the pool from which brain DHA is ostensibly derived. We wonder therefore how raising dietary DHA would raise brain DHA. The inconsistencies between low DHA intake but possibly normal plasma and brain DHA in AD, and possible efficacy of supplemental DHA in AD may be more methodological than biological, and may arise in part because only one study to date has reported both DHA intake and plasma DHA values in the same AD patients. Studies reporting DHA intake and plasma levels while also undertaking a DHA intervention in AD would presumably help resolve these issues.

O11

FKH1P, FKH2P AND THE ANAPHASE PROMOTING COMPLEX PARTNER ON CONTROLLING CHRONOLOGICAL LIFESPAN IN SACCHAROMYCES CEREVISIAE.

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The yeast Anaphase Promoting Complex (APC) is required for cell cycle progression, genomic stability and extended lifespan, and is inhibited by glucose signaling. Glucose signals a switch in yeast whereby rapid growth is promoted over stress response and genome maintenance. In higher eukaryotes the FOXO stress response transcription factors are required for extended lifespan and are turned off under favorable conditions. In this report, we asked whether the yeast FOXO proteins Fkh1p and Fkh2p were required for stress response and lifespan, and whether this involved the APC. Deletion of both *FKH1* and *FKH2* was required to exacerbate the temperature sensitive (*ts*) growth phenotypes of *apc5^{CA}* and *apc10Δ*. In fact, *apc11-13 fkh1Δ fkh2Δ* mutants could not be isolated. We also demonstrated that *apc5^{CA}* and *fkh1Δ fkh2Δ* mutants were sensitive to oxidative stress, with the *apc5^{CA} fkh1Δ fkh2Δ* triple mutant hypersensitive. Fkh1p and Fkh2p are believed to activate the transcription of histones and several APC activators. As expected, we found that histone protein levels were reduced in *fkh1Δ fkh2Δ* cells. However, in line with a role for the APC in histone deposition, the *apc5^{CA} fkh1Δ fkh2Δ* mutant had dramatically reduced histone levels. These phenotypes are associated with reduced chronological lifespan in *fkh1Δ fkh2Δ* and *apc5^{CA} fkh1Δ fkh2Δ* cells. Exposure to 25 mM H₂O₂ dramatically reduced the lifespan of the triple mutant, indicating that the APC and the Fkh proteins work in parallel pathways to respond to oxidative stress. Consistent with the idea that the Fkh proteins activate the APC, low-level expression of *FKH1* suppressed the *ts* phenotypes of most mutant APC subunits, but was toxic to all cells when over expressed. These observations identify an evolutionarily important mechanism used to control lifespan-dependent APC activity.