
LETTER FROM THE CSLSR PRESIDENT

It is with great pleasure that I announce the collaboration of the Canadian Society for Life Science Research (CSLSR) and the *McGill Journal of Medicine* for the publication of the student abstracts from the CSLSR's 2nd Annual Conference held at McGill University on July 13th and 14th, 2007.

The CSLSR is a nationally registered non-profit organization created by life science students for fellow student researchers. We are dedicated to bringing together young student researchers at the undergraduate, graduate, post-graduate and professional levels to share upcoming scientific/health research and discoveries, and allow for furthering the knowledge amongst these future academics, clinicians, clinician-scientists and industry professionals in the life science fields.

During the conference, we were honoured to welcome renowned scientist keynote speakers from both academia and industry. For a synopsis of the conference, and for more information on our upcoming events and student opportunities, please visit our website at www.cslsr.ca.

On behalf of the executive and chapter representatives of the CSLSR, I would like to thank the Canadian Institutes of Health Research (CIHR)'s Institute of Infection and Immunity and Merck-Frosst for their support of this year's conference. We gratefully acknowledge McGill University for their support in our hosting of this year's conference at their institution. I would also like to personally congratulate the student oral and poster presentation award winners:

Poster Presentations:

1. Rozanne Arulanandam - Queen's University
2. William Montgomery McKillop - University of Western Ontario
3. Reva Vidia Mohan - Queen's University

Oral Presentations:

1. Safaa Sebak - McGill University
2. Stephen Andrews - McGill University
3. Dominic Paquin Proulx - Université Laval

Finally, we would like to thank all attendees, speakers, affiliates and sponsors from across the country for making this conference a great success. We look forward to seeing you in 2008.

Thank-you for your support of young researchers at McGill and across Canada!

Philippe Rizek
President

Canadian Society for Life Science Research/
Société Canadienne de Recherche des Sciences de la Vie
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The Soldier and the Animal: metaphor in maintenance of antiretroviral adherence in Ghana's Eastern Region

MATTHEW J. AKIYAMA AND DAVID NAPIER
DEPARTMENT OF ANTHROPOLOGY, UNIVERSITY COLLEGE LONDON

Background: Adherence is of the utmost importance in human immunodeficiency virus (HIV) antiretroviral therapy (ART). Poor adherence leads to viral resistance and increases the likelihood of treatment failure. As ART becomes increasingly available in the developing world, differences in cultural understandings of illness and language pose specific challenges to maintaining adherence. **Objective:** To identify cultural and linguistic barriers to treatment comprehension and the successful strategies used to achieve adherence in a population of people living with HIV/AIDS (PLWHA) in rural Ghana. **Methods:** Fieldwork was carried out in a Krobo community in Ghana's Eastern Region. In depth, semi-structured, and open-ended interviews were conducted with PLWHA in the early stages of ART. Observations during clinic visits and support group sessions for PLWHA were made. Focus group discussions were held with clinical staff, community leaders, and PLWHA representatives. **Results:** An understanding of the relationship between the immune system, HIV, and ART was crucial for establishing adherence in PLWHA. This relationship was negotiated organically by PLWHA and health care providers in the early stages of treatment and resulted in a set of mutually agreed upon metaphors to draw from during the therapeutic process. For example, the globally utilized metaphor of 'soldiers' as white blood cells defending the body against foreign invaders was used to illustrate the role of the immune system. The local term 'lowii' - the Krobo word for animal - was used to represent the virus. Metaphors used to characterize the role of ART in viral suppression were perhaps the most important. Many believe they are not sick in the latent phase of the illness since symptoms are not yet present. Therefore adherence was promoted using metaphorical scenarios such as applying constant pressure on a bowl to prevent the escape of a snake from beneath it. **Conclusions:** Metaphoric amalgamations of local cosmology with biomedical representations of the immune system, HIV and ART are instrumental to the way in which complex ART regimens are grappled with and ART adherence is maintained. The descriptive findings in this study may be of use in other treatment settings where cultural and language barriers persist.

Keywords: HIV, antiretroviral therapy, adherence, Ghana, Krobo, illness narrative

Lipid metabolism in peroxisomes, endoplasmic reticulum and lipid bodies controls chronological aging in yeast

S. BOURQUE, A. GOLDBERG, T. BOUKH-VINER, C. GREGG, P. KYRYAKOV, S. CHOWDHURY AND V.I. TITORENKO
DEPARTMENT OF BIOLOGY, CONCORDIA UNIVERSITY, MONTREAL, QUEBEC, CANADA

The diet known as calorie restriction (CR) extends life span and delays diseases of aging. It seems that the fundamental mechanisms of aging and the stimulatory effect of CR on life span are conserved from yeast to humans. We use yeast as a model organism for studying the molecular and cellular mechanisms of aging. Our research is aimed at understanding how defects in the biogenesis and function of the peroxisome, an organelle known for its essential role in lipid metabolism, affect the life span of yeast placed on the CR diet. We found that CR promotes the lipolysis of neutral lipids (NL) stored in lipid bodies (LB). In addition, the CR diet stimulates the synthesis of peroxisomal enzymes involved in oxidation of the LB-derived CoA esters of fatty acids (FA-CoA), thereby leading to the rapid consumption of free fatty acids (FFA) in chronologically aging cells. Using lipidomics, we monitored the dynamics of the age-related changes in the intracellular levels of NL, FFA, diacylglycerols (DAG) and cardiolipins (CL) in numerous long- and short-lived mutants. Our findings imply that the mobilization of FA-CoA from LB and their subsequent oxidation in peroxisomes play a key role in regulating chronological life span and in protecting CR yeast from caspase- and mitochondria-dependent apoptosis and various stresses. Moreover, our data provide evidence that the steady-state levels of the LB-derived FA-CoA and of the LB- and endoplasmic reticulum (ER)-derived DAG control the rate of chronological aging. Furthermore, our findings suggest that lipid metabolism in peroxisomes of CR yeast modulates the steady-state levels of CL in the inner mitochondrial membrane. This, in turn, regulates a distinct set of processes that take place in mitochondria. Our data suggest a mechanism by which the remodeling of lipid metabolism in peroxisomes, ER and LB of CR yeast extends their life span by regulating the apoptosis- and stress response-related mitochondrial functions.

Activation of global DNA demethylation by MBD2 converts non-transformed cells into highly invasive and metastatic cancer cells; MBD2 mediates RAS transforming activity

S.D.ANDREWS, B. ATEEQ, J. TORRISANI, A.C.D'ALLESIO, A. UNTERBERGER, J-N OU, A. MCKINNEY, S. RABBANI, AND M. SZYF

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, FACULTY OF MEDICINE, MCGILL UNIVERSITY, MONTREAL, QUEBEC, CANADA

Introduction: Cancer cells are hallmarked by global DNA hypomethylation and regional promoter hypermethylation. The latter has been extensively studied while insufficient attention has been directed at global DNA hypomethylation. MBD2 is the only protein that has been implicated in removal of methyl groups from CG dinucleotides. Inhibition of MBD2 attenuates tumorigenesis, metastasis, and reverses the hypomethylation of metastatic genes. Additionally, germ line deletion of the *mbd2* gene protects mice from intestinal tumors. Therefore, we tested the hypothesis that oncogenic signals induce MBD2 resulting in metastatic transformation of cells through the triggering of global DNA hypomethylation. **Results:** Activated HA-RAS expression induced MBD2 protein levels and induced global DNA hypomethylation. Overexpression of MBD2, but not a mutant MBD2, converted untransformed mouse fibroblast and human epithelial cells into highly transformed metastatic cells with hypomethylated genomes. Cells overexpressing MBD2 form tumors in Nude mice and invade and degrade bone in SCID mice. Consistent with the hypothesis that MBD2 plays a critical role in oncogenic transformation, MBD2 knockdown in HA-RAS transformed cells attenuates transformation and increases DNA methylation. Remarkably, this phenotypical reversal by MBD2 knock down was rescued by ectopic expression of wt MBD2 but not mutant MBD2. Thus, our data suggests that MBD2 mediates part of the oncogenic and prometastatic activity of Ras. To determine if MBD2 induces oncogenic and metastatic transformation by inducing gene expression through hypomethylation, Affymetrix expression arrays were used and revealed that MBD2 induced a panel of genes involved in metastatic transformation. ChIP assays and methylation analyses confirmed that several of these genes were induced through MBD2 binding and demethylation. Finally, knockdown of HA-RAS in a human cancer cell line with an activated RAS mutation, T24, resulted in decreased: MBD2, DNA demethylation, cellular invasion, and gene expression of metastatic genes. The same effect was shown when MBD2 was decreased in T24 cells but not when only the metastatic genes were knocked down. **Summary:** Our results have unraveled a critical transformation pathway leading from RAS through MBD2 to global hypomethylation and activation of metastatic genes. These data support a new therapeutic approach to metastasis, which would involve targeting MBD2 and DNA demethylation. This work was supported by a grant from the National Cancer Institute of Canada to MS.

Keywords: metastasis, epigenetics, demethylation, RAS activation, oncogenic signaling

Influence of oral and subcutaneous bisphenol-A on intrauterine implantation of fertilized ova in inseminated female mice

ROBERT G. BERGER, TRINA HANCOCK, DENYS DECATANZARO

Intrauterine implantation of fertilized ova in inseminated females is sensitive to minute levels of natural estrogens. Bisphenol-A (BPA), a widely used chemical in the production of polycarbonate plastics and epoxy resins, can be estrogenic. Here we administered BPA during the period of implantation to determine levels of exposure required to terminate pregnancy in mice. Varied doses were given through either injection or ingestion. Subcutaneous injections during days 1-4 of gestation significantly reduced litter size at 3.375 mg/day and substantially reduced the proportion of females that were parturient at 10.125 mg/day. Uterine implantation sites were significantly reduced at a dose of 10.125 mg/day whereas a dose of 6.75mg/day completely inhibited implantation. Exposure to lower doses was without significant effect. When inseminated females' diets were supplemented on days 1-5 with peanut butter contaminated by 0.11-9.0% BPA, litter size and percent parturient were not affected. However, when the animals' diet was exclusively comprised of mixture of BPA, peanut butter, and powdered chow during days 1-4, an average daily intake of 68.84mg BPA terminated all pregnancies. No significant effects at lower doses of BPA were seen in number of births or other measures through either mode of administration.

Keywords: Bisphenol-A, implantation, estrogens, pregnancy, uterus

Regulation of the Breast Cancer Susceptibility Gene 1 (BRCA1) by the Stress Hormone Hydrocortisone

LILIA ANTONOVA AND CHRISTOPHER R. MUELLER
CANCER RESEARCH INSTITUTE, QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, CANADA

Psychological stress has been correlated with breast cancer development in numerous epidemiological studies. However, few physiological and molecular models which may account for this association are available. We have found that the stress hormone hydrocortisone down-regulates the expression of the breast cancer susceptibility gene BRCA1 in non-malignant mouse and human mammary cells. Since low levels of BRCA1 have been implicated in the development of sporadic breast cancer, this may represent a novel molecular mechanism through which stress signaling disrupts intracellular pathways involved in the prevention of tumorigenesis. The hydrocortisone effect on BRCA1 was observed at both the promoter and mRNA levels, and was found to be concentration-dependent and reliant on continuous hydrocortisone presence. Also, it is unchanged by the addition of lactogenic hormones, or growth conditions. Hydrocortisone was also found to negate a known positive effect of estrogen on BRCA1 expression and therefore, may interfere with estrogen-related signaling in mammary epithelial cells. The repressive effect of hydrocortisone is lost in malignant mouse and human mammary cells, suggesting alteration of signaling to the BRCA1 promoter in the course of cell transformation. We have uncovered two promoter regulatory sites, which are involved in BRCA1 regulation by hydrocortisone, the BRIBS and UP regulatory elements. Binding of the transcription factor GABP α/β to both sites and binding of the transcription factor USF2 to the UP site are lost upon hydrocortisone addition, suggesting the involvement of MAPK signaling in hydrocortisone-induced repression. Interestingly, we have identified a direct role of the glucocorticoid receptor (GR) in BRCA1 regulation in the absence of hydrocortisone which is independent of GR DNA binding ability. Thus, GR may act as an activator of BRCA1 expression, which is recruited away from the BRCA1 promoter along with its protein binding partners in periods of stress.

Breast cancer cells inhibit osteoblast differentiation

JENNA FONG
FACULTY OF DENTISTRY, MCGILL UNIVERSITY, MONTRÉAL, QUÉBEC, CANADA

Skeletal metastasis is a major complication of advanced breast cancer which results in bone fractures and considerable pain burden. Osteoclasts recruited to the site of metastasis lead to the destruction of bone and formation of osteolytic lesions. Osteoblasts are known to contribute to osteoclast activation by producing a key pro-resorptive cytokine RANKL, however the effects of cancer cells on osteoblasts are not fully understood. We have studied the effects of MDA-MB-231 human breast carcinoma cells on proliferation and differentiation of primary osteoblast precursors. MDA-MB-231 cells were cultured until 80% confluent, and conditioned medium was collected after 24h of culturing. Bone marrow cells were isolated from the long bones of C57BL/6J mice, and treated for 5 days with ascorbic acid (50 mg/ml) in the presence or absence of MDA-MB-231 conditioned medium (10%). As a control, we used medium conditioned by MC3T3-E1 cells. Cell numbers were assessed in samples labeled with nuclear stain DAPI, and processed using image analysis. Treatment with ascorbic acid alone led to formation of 390 +/- 90 cells/mm². Addition of MDA-MB-231 conditioned medium to osteoblastic cultures led to a 2-3 fold increase in cell number, whereas treatment of cultures with MC3T3 conditioned medium had no effect, indicating that MDA-MB-231 cells produce factors that stimulate proliferation of osteoblastic cells. To assess osteoblast differentiation, the cultures were stained for alkaline phosphatase (ALP) and the labeled area was quantified using image analysis. Even at this early cultures, treatment with ascorbic acid alone led to significant increase in ALP-positive cells, compared to untreated cultures. Addition of MDA-MB-231 conditioned medium to ascorbic acid-treated cultures resulted in a 5-fold decrease in ALP expression. Thus, our data indicate that soluble factors produced by breast cancer cells stimulate proliferation and inhibit differentiation of native osteoblasts. Since it has been previously shown that immature osteoblast precursors represent a major source of RANKL, compared to mature osteoblasts, our findings suggest a novel mechanism for osteoblast-mediated stimulation of osteoclasts that potentially contributes to the establishment and progression of metastatic lesions in bone.

Heat shock cognate protein 70 accumulates in the nucleolus of HeLa cells during heat stress recovery

PIOTR BAŃSKI, MOHAMED KODIHA AND URSULA STOCHAJ

DEPARTMENT OF PHYSIOLOGY, MCGILL UNIVERSITY, MONTREAL, QUEBEC, CANADA

Heat shock cognate protein 70 is a constitutively synthesized chaperone involved in many cellular functions, such as in the proper folding of proteins, protein targeting to various organelles, refolding of damaged proteins and in the targeting of severely damaged proteins to the degradation pathway. It therefore prevents the formation of dangerous protein aggregates and is necessary to cell survival. Hsc70s are involved in multiple physiological mechanisms including aging, cancer development, ischemia of the heart and brain, in the response to stress such as heat shock and other environmental changes.

Under normal growth conditions, hsc70 is present throughout the cell. Following heat exposure, hsc70s accumulate in the nucleoplasm and concentrate transiently in nucleoli when cells recover from stress through unknown mechanisms. To define those mechanisms, I determined the kinetics of hsc70 nucleolar accumulation in human culture cells that recover from stress. The molecular signaling events that play a role in hsc70 nucleolar accumulation have yet to be defined. My results demonstrate that several kinase cascades and phosphatases contribute to the control of hsc70 nucleolar accumulation in cells recovering from stress. Furthermore, I have shown that hsc70s are present in high molecular mass complexes in nucleoli of both heat-shocked and control cells. However, the size and possibly composition of these complexes changes in response to stress; the binding partners that associate with hsc70 in nucleoli are currently being identified.

To identify regions of hsc70 that are necessary and sufficient for transport to the nucleolus, constructs were generated that contain defined portions of hsc70 fused to EGFP. Using confocal microscopy, I identified a short segment of hsc70 that is sufficient for targeting a non-nucleolar passenger protein to the nucleolus of stressed cells. My research is now defining the minimal region sufficient for stress-induced nucleolar targeting.

I expect my studies to contribute to the understanding of the dynamic localization of hsc70s and the signaling events that control these reactions. Using a combination of biochemistry, cell and molecular biology, my research will provide new insights into the stress response and, in particular, the physiological roles of hsc70s in nucleoli of stressed cells.

Keywords: Hsc70, nucleoli, stress, accumulation, nucleolar targeting

Probing into the GTP specificity of an mRNA capping enzyme

ISSUR MOHESHWARNATH AND MARTIN BISAILLON

DÉPARTEMENT DE BIOCHIMIE, UNIVERSITÉ DE SHERBROOKE, SHERBROOKE, QUEBEC, CANADA

Most cellular and some viral mRNAs contain at their 5' ends a cap structure, which is essential for the mRNA stability, its nucleo-cytoplasmic transport and its translation. The synthesis of this cap structure, a 7-methyl guanosine residue linked via a 5'-5' triphosphate bridge to the RNA transcript, is carried out co-transcriptionally via three sequential enzymatic reactions, the first one involving the hydrolysis of the RNA 5' triphosphate terminus by an RNA triphosphatase to form a diphosphate end, the second step being the transfer of a GMP moiety to the diphosphate end of the RNA by an RNA guanylyltransferase activity which is followed by the methylation of the GpppN cap at position N7. The RNA guanylyltransferase belongs to the nucleotidyltransferase superfamily, which also includes ATP dependent DNA ligases. Despite sharing the same conserved motifs, each enzyme uses different substrates for its activity. The aim of this research is to probe into the molecular determinants for the GTP specificity of the model RNA guanylyltransferase from PBCV-1 (the smallest known enzyme of this family) by gaging its effectiveness to use various purine analogs as substrate. In addition to providing an insight into the capping mechanism this approach will also enable the determination of molecular specificities for the methylation of the cap structure.

Analysis of DAF-18/PTEN in VAB-1 Eph RTK Signaling

SARAH E. BRISBIN AND IAN D. CHIN-SANG
QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, CANADA

The elucidation of signaling pathways involved in cell movements is critical for understanding complex developmental processes. Accordingly, consideration must be given to the function of the individual components of such signaling cascades. DAF-18, a *C. elegans* homolog of the PTEN human tumor suppressor, is a component of the DAF-2 Insulin Receptor-like signaling pathway involved in dauer formation, longevity and metabolism. Our lab has shown that DAF-18 can physically bind VAB-1, an Eph Receptor Tyrosine Kinase with roles in axon guidance and embryonic morphogenesis. To corroborate these varied functions as well as explore additional roles of DAF-18 in the worm, affinity purified DAF-18 antibodies were used for in situ detection of DAF-18 as well as Western blotting. Results of Western blotting confirm that DAF-18 is present in N2 as well as our over-expressing lines at the expected molecular weight of 110 kDa but is absent in *daf-18* (*ok480*), suggesting this represents a molecular null allele. Here we show that DAF-18 is expressed in amphid neurons, ventral nerve cord, nerve ring and the germline precursor cells, Z2 and Z3. The presence of DAF-18 in neuronal tissues is consistent with the known expression pattern of VAB-1, further substantiating an in vivo interaction. Additionally, we have shown endogenous VAB-1 is also expressed in the Z2 and Z3 germline precursors. Previous literature implicates VAB-1 in regulating oocyte maturation, suggesting expression in the germline. DAF-18 has recently been implicated in regulating germline proliferation in L1 arrest, and our results are consistent with DAF-18 functioning in the germline. While the exact mechanism of the DAF-18/VAB-1 interaction requires further investigation, preliminary molecular evidence proposes VAB-1 may be a negative regulator of DAF-18. DAF-18 has been shown to exhibit PIP3 lipid phosphatase activity and we are currently testing whether DAF-18 has protein phosphatase activity on VAB-1. Taken together these results support the in vivo interaction of DAF-18 and VAB-1 which we will be exploring further with 4D analysis to ascertain the role of *daf-18* in embryonic morphogenesis, double mutation analysis, aging and dauer studies.

Keywords: VAB-1 Eph Receptor, DAF-18/PTEN, *C. elegans*, tumour suppressor, antibodies

Megakaryocytic cells expressing a peptide derived from a protein regulating the actin cytoskeleton, MTPG-24, exhibit an increased cell size

A. STE-MARIE^{1,2}, C. SIMARD¹ AND S. CÔTÉ^{1,2}
¹HÉMA-QUÉBEC, QUÉBEC, QUEBEC, CANADA. ²DÉPARTEMENT DE BIOCHIMIE ET MICROBIOLOGIE, UNIVERSITÉ LAVAL, QUÉBEC, QUEBEC, CANADA

Megakaryocytes (MKs) are specialized hematopoietic cells that produce blood platelets as a result of the fragmentation of their cytoplasm during the last phase of their life. Prior to this event, MKs undergo a complex maturing process during which they accumulate large nuclear mass and cytoplasmic cellular volume. The large size of the MKs is associated with extensive polyploidization, involving multiple rounds of incomplete mitosis termed endomitosis. There is now accumulating evidence that endomitotic MKs develop as a result of aberrant regulation of cleavage furrow formation, to which is normally associated a localized production of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) during the creation of membrane barriers between two daughter cells. Here we report that human megakaryocytic cell lines over-expressing transgenic constructs encoding the MTPG-24 peptide undergo remarkable changes in cellular size, resulting in cells with the appearance of mature MKs. MTPG-24 is a 24mer-peptide with PI(4,5)P₂ binding affinity which derives from a protein involved in regulating actin-based structures and motility. MTPG-24 tagged with fluorescent proteins was detected closely associated to the plasma membrane where it accumulated in a patchy pattern, in addition to strongly co-localizing with a Golgi marker. Since no significant differences in cellular proliferation nor in DNA content were observed between control cells and those expressing the peptide, the increased cell size exhibited by MTPG-24-expressing cells, in contrast to MKs, does not result from endomitosis. Because it likely interferes with actin cytoskeleton regulatory protein by masking membrane PI(4,5)P₂, MTPG-24 may thus provide an interesting tool to modulate cell shape and size. We are currently testing whether this peptide could be used to increase the in vitro platelet production of human cord blood-derived MKs which have a low propensity to become polyploid.

Keywords: hematopoiesis, blood platelet production, megakaryocytes, polyploidy, cell division

Effect of sensori-motor interventions on the oral feeding performance of preterm infants

S FUCILE MSc OTR^{1,2}, E GISEL PhD OTR ERG¹, C LAU PhD²

¹MCGILL UNIVERSITY, MONTREAL, CANADA. ²BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX, USA

Background: Up to 30-40% of preterm infants may encounter oral feeding difficulties. Successful oral feeding is a complex process necessitating the appropriate function and integration of multiple systems, including oral, cardio-respiratory, gastrointestinal and neurological. Oral feeding difficulties may ensue from oral and non-oral origins. At present, most sensori-motor interventions are aimed at improving oral-motor skills only. Limited information is available on the effect of non-oral sensori-motor interventions on preterm infants' oral feeding performance.

Objective: To assess whether oral (O), tactile/kinesthetic (T/K), and combined (O+T/K) stimulations enhance the oral feeding performance of preterm infants, and to elucidate the impact of multiple sensori-motor stimulations on oral feeding outcomes. **Methods:** Seventy-five preterm infants (< 32 weeks gestation) were randomly assigned to an O, T/K, combined (O+T/K), or sham (S) group. The O intervention consisted of stroking the lips, gums, tongue and sucking on a pacifier. The T/K intervention involved stroking the head, trunk, limbs and passive range of motion to limbs. The combined (O+T/K) was similar to the above. The S intervention involved observing the infant only. Interventions were administered for 10 days, 2X/day, for 15 minutes. The outcome measures included 1) number of days from introduction to independent oral feeding, 2) volume transfer (%volume taken/prescribed volume), and 3) rate of transfer (ml/min). Outcome measures were monitored when infants were taking 1-2, 3-5, and 6-8 oral feedings/day. One-way ANOVA and repeated measures 2-way ANOVAs were applied. **Results:** Independent oral feeding was achieved significantly earlier by the O (11.1±3.5), T/K (11.4±3.3), and combined (O + T/K) (10±3.5) groups than the control group (20.7±6.6), $p < 0.000$. Volume transfer ($p = 0.000$) and rate of transfer ($p = 0.003$) were significantly greater in the 3 experimental vs. control group. The 3 sensori-motor interventions had similar oral feeding outcomes. **Conclusion:** The O, T/K, and combined (O+T/K) interventions accelerated the transition from tube to independent oral feeding to the same degree. This was associated with enhanced volume transfer and rate of transfer observed in all 3 experimental groups. The results support the concept that development of oral feeding skills may be influenced by early sensori-motor experiences beyond the boundaries of the oral system.

Complementary role for TC-PTP and PTP-1B in interferon-gamma signalling

ANNIE BOURDEAU^{1*}, KRISTA M. HEINONEN^{1,2*}, KAREN M. DOODY^{1,3}, EMILY K. HIGGINS¹, AILSA LEE-LOY¹, MICHEL L. TREMBLAY^{1,3}

¹MCGILL CANCER CENTRE, ²DIVISION OF EXPERIMENTAL MEDICINE, MCGILL UNIVERSITY, ³DEPARTMENT OF BIOCHEMISTRY, MCGILL UNIVERSITY, MONTRÉAL, QUÉBEC, CANADA

*Both authors have contributed equally to the work, co-first authors

The control of tyrosine phosphorylation depends on the fine balance between kinase and phosphatase activities. Protein tyrosine phosphatase 1B (PTP-1B) and T cell protein tyrosine phosphatase (TC-PTP) are two closely related phosphatases that are known to control cytokine signalling, for example, in macrophages. We wished to study the redundancy of PTP-1B and TC-PTP on interferon signalling by deleting one or both copies of PTP-1B in *tcptp*^{-/-} and *tcptp*^{+/-} mice by interbreeding. Our results indicated that the double mutant was lethal at a relatively early stage of embryonic development. Mice heterozygous for TC-PTP on the *ptp1b*^{-/-} background developed symptoms similar to a chronic inflammatory disease, and their macrophages were highly sensitive to interferon- γ as shown by increased Stat1 phosphorylation and nitric oxide production. Together, these data indicate a nonredundant role for PTP-1B and TC-PTP in the regulation of interferon signalling.

Keywords: T cell protein tyrosine phosphatase, protein tyrosine phosphatase-1B, interferon-gamma, inflammation, cell signalling

Inhibition of hsc70s shuttling upon stress, import, export, and beyond?

MOHAMED KODIHA, PIOTR BANSKI, AND URSULA STOCHAJ

DEPARTMENT OF PHYSIOLOGY, MCGILL UNIVERSITY, MONTREAL, QUEBEC, CANADA

Heat shock proteins are molecular chaperones that are involved in multiple cellular processes. This includes repair of stress induced damage and targeting of proteins to different organelles in unstressed cells. Under normal growth conditions cytoplasmic members of the hsp/hsc70 family shuttle between nuclei and cytoplasm and they are found in both compartments. Upon exposure to stress, hsc70s accumulate in nuclei of stressed cells and heat shock is the most efficient treatment to induce their nuclear accumulation. Although heat increases the steady-state concentration of hsc70s in nuclei, it is not known whether stress also controls their ability to shuttle between nuclei and cytoplasm. To gain more insight into the effect of stress on hsc70s shuttling, and the molecular mechanisms which control this process, we have analyzed hsc70s trafficking in heat-stressed human/mouse heterokaryons. We found that heat shock inhibits hsc70s shuttling and sequesters the chaperone in nuclei. However this stress-induced shuttling inhibition is transient only as hsc70s were able to resume shuttling when cells recover from heat. We have defined nuclear retention as one of the mechanisms which prevent hsc70s exit from nuclei, and leads to shuttling inhibition in stressed cells. This retention depends on two forms of hsc70 interaction with nuclear anchors, ATP-sensitive binding of hsc70s to chaperone substrates and ATP-insensitive association with nucleoli. Our results showed increase in the association between hsc70s and nucleolar proteins fibrillarlin and rpS6 upon stress, in line with the idea that hsp/hsc70s protect the nucleoli from stress induced damage. Taken together, our studies show that heat stress increases the retention of hsc70s in nuclei which prevents the exit of the chaperone to the cytoplasm and therefore inhibits shuttling. We propose that upon recovery, hsc70 is liberated from nuclear and nucleolar anchors and this is a prerequisite to resume shuttling.

Keywords: Hsc70s, nucleus, shuttling, stress, heterokaryons

Spotlights on the DNA repair system in late spermatogenesis: stage-specific DNA fragmentation and activation of H2AX

F. LEDUC, V. MAQUENNEHAN, G. BIKOND NKOMA AND G. BOISSONNEAULT

DÉPARTEMENT DE BIOCHIMIE, FACULTÉ DE MÉDECINE, UNIVERSITÉ DE SHERBROOKE, SHERBROOKE, QUEBEC, CANADA

Introduction: The histone variant H2AX is involved in early DNA damage response and in the recruitment of repair proteins at sites of double-strand breaks. In testis, the active form of H2AX (γH2AX) is thought to be involved in two processes namely the inactivation of sex chromosomes and the control of genome integrity during meiotic recombination. We hypothesized that H2AX is also activated during spermiogenesis, when 100% of elongating spermatids (ES) harbor transient DNA breaks. **Material and methods:** We used confocal and epifluorescence microscopy applied to both immunofluorescence and Terminal deoxynucleotidyl transferase dUTP nick-end labeling on squash preparations of seminiferous tubules and fixed mouse testis sections. **Results:** We demonstrate the presence γH2AX foci in ES, coincident with the onset of transient DNA strand breakage and chromatin remodeling as shown by the hyperacetylation of histone H4. Topoisomerase IIb is also present in foci in ES and may be involved in the DNA fragmentation and activation of H2AX. Furthermore, we were found the tyrosyl DNA phosphodiesterase 1 (TDP1), an enzyme known to remove topoisomerase adducts. In addition, in-situ incorporation of dUTP-FITC clearly shows that an active DNA polymerase is present. **Conclusions:** These results demonstrate for the first time that a complex DNA repair system is required during the chromatin remodeling in ES and likely essential to the genomic integrity of male gametes. Given the haploid character of ES that cannot rely on homologous recombination for DNA repair, we hypothesize that the error-prone non-homologous end-joining (NHEJ) mechanism is present in late spermiogenesis. Faulty repair of the NHEJ may be involved in male infertility and bear dramatic consequences for offspring. Not surprisingly, alterations in the nuclear integrity of the male gametes have been associated with de novo genetic disorders, developmental and morphological defects, cancer and miscarriage. Funded by the Canadian Institutes of Health Research (grant #MOP-74500)

Keywords: spermiogenesis, chromatin, DNA repair, genomic integrity, topoisomerase

Murine model for implant osseointegration

LETITIA Z. LIM¹, S. A. HACKING², A. LI¹, H. WANG¹, E. J. HARVEY², J. E. HENDERSON¹

DEPARTMENTS OF ¹MEDICINE AND ²SURGERY, FACULTY OF MEDICINE, MCGILL UNIVERSITY, MONTRÉAL, QUEBEC, CANADA

Rationale: Bone regeneration decreases with advancing age in humans. Consequently, there is an increased risk of failure to achieve cementless implant fixation by bone attachment in the elderly. Individual and age-related differences in bone regeneration are believed to arise in large part from genetically programmed pathways that regulate the response of osteogenic cells to growth factors and to surface topography. We used mice deficient in growth factor signaling as a model to study bone regeneration in response to different surface textures. **Methods:** Femoral implants with smooth (Sm) or micro-textured (μ Tx) surfaces were fabricated and coated with titanium to generate a bio-inert surface. Mice deficient in FGF (FGFR3^{-/-}) and PTHrP (PTHrP^{+/-}) signaling were used as in vivo models to examine bone regeneration. Bone was quantified in vivo using micro-CT and classic histology. **Results:** Bone regeneration was enhanced around Tx femoral implants compared with Sm implants and was decreased in PTHrP^{+/-} and FGFR3^{-/-} mice compared with wild type littermates. An extensive layer of fibrous tissue was seen apposed to the Sm implant in FGFR3^{-/-} mice. **Conclusion:** Impaired growth factor signaling in vivo resulted in decreased bone formation and fibrous tissue formation, similar to that commonly seen in aged humans who have undergone total joint arthroplasty. Combined with results from in vitro assays, this approach represents a simple, biologically relevant model to screen for combinations of surface texture and biologic agents to promote bone regeneration in the elderly bone regeneration decreases with advancing age in humans.

Funding: CIHR, RRBO, RRTQ

Keywords: osteoporosis, bone regeneration, tissue engineering, implant osseointegration

Interleukin 33 (IL-33) in severe asthma and modulation of its expression in airway smooth muscle cell (ASMC)

D. PREFONTAINE¹, S. LAJOIE-KADOCH¹, A.K. MOGAS¹, S. FOLEY¹, R. OLIVENSTEIN², A.J. HALAYKO³, P. ERNST⁴, C. LEMIERE⁵, J.G. MARTIN¹, Q. HAMID¹

¹MEAKINS-CHRISTIE LABORATORIES, MCGILL UNIVERSITY. ²MONTREAL CHEST RESEARCH INSTITUTE, MCGILL UNIVERSITY. ³UNIVERSITY OF MANITOBA. ⁴MCGILL UNIVERSITY; ⁵SACRÉ-COEUR HOSPITAL, UNIVERSITY OF MONTREAL

Rationale: IL-33, a recently described IL-1 cytokine family member, promotes Th2 inflammation but evidence on implications of this cytokine in asthma is lacking. IL-33 is mainly expressed by structural cells including ASMC, but whether pro-inflammatory cytokines modulate its expression in ASMC is unknown. **Methods:** RNA was extracted from endobronchial biopsies collected from adults with mild (n = 8), moderate (8), severe (9) asthma and from control subjects (5). Reverse transcriptase and real-time quantitative PCR were used for determining IL-33 transcript levels. ASMC isolated from pathologically uninvolved bronchial airway segments of resected lung specimens were cultured with or without TNF α , and the effects of IFN γ , Dexamethasone (DEX) or Mithramycin A (MMA) additions were investigated. **Results:** Higher levels of IL-33 transcripts were detected in biopsies from asthmatic patients (mild, moderate and severe) compared to control subjects (p = 0.932, 0.0186 and 0.002, respectively). In ASMC, TNF α upregulated IL-33 mRNA in a time- and dose-dependent manner. IFN γ synergized with TNF α -induced upregulation of IL-33. MMA reduced the TNF α -induced IL-33 upregulation, whereas DEX did not display significant effect. **Conclusion:** IL-33 expression was shown to increase with asthma severity in bronchial biopsies. In cultured ASMC, MMA reduced the TNF α -induced IL-33 upregulation, whereas DEX had no effect. Although IL-33 was shown to promote eosinophilia, Th2-type phenotype and cytokines, our data suggest that IFN γ induces IL-33 expression in ASMC.

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Keywords: asthma, inflammation, interleukin 33, bronchial biopsies, airway smooth muscle cells

Characterization of CD11d leukocyte integrin surface expression

W. M. MCKILLOP AND G. A. DEKABAN

ROBARTS RESEARCH INSTITUTE, DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY, UNIVERSITY OF WESTERN ONTARIO, ONTARIO, CANADA

Primary spinal cord injury (SCI), a mechanical trauma to the spinal cord, results in death to neurons and activation of various cellular responses that contribute to secondary SCI and further neuronal loss. The most potent of these cellular responses is the inflammatory response at the site of injury involving cells resident in the spinal cord, as well as infiltrating leukocytes. As leukocytes leave blood vessels they release pro-inflammatory molecules, including free radicals in the form of an oxidative burst. These free radicals cleanse the area of potential infectious agents, but also contribute to cell death. Previous studies have shown that this cellular infiltration of leukocytes into a site of inflammation involves the CD11d/CD18 integrin as monoclonal antibodies to CD11d block this process and result in reduced secondary SCI and improved neurological recovery. Very little is known about the CD11d/CD18 integrin itself other than that extensive surface expression of the protein is limited to leukocytes at regions of local inflammation. Our study focuses on identifying the mechanisms that regulate CD11d surface expression on leukocytes. CD11d is a member of the leukocyte-specific $\beta 2$ family of integrins. Other members of this family (CD11a,b,c) require heterodimerization with CD18 for surface expression. Thus it is expected that CD11d will also be expressed on the surface of leukocytes expressing both CD11d and CD18. An analysis of the human CD11d amino acid sequence revealed a potential Casein Kinase II (CKII) phosphorylation motif. Numerous proteins have their cellular localization controlled by CKII phosphorylation, but no integrin has previously been reported to contain an active CKII phosphorylation site. We hypothesize that CKII phosphorylates CD11d, and that this phosphorylation contributes to the complex regulation of the intracellular distribution of the CD11d/CD18 integrin. Our specific aims are to determine if prior formation of a CD11d/CD18 heterodimer is required for CD11d to localize to the cell surface, if CKII phosphorylates CD11d, and to elucidate how the interplay between these two factors relates to CD11d surface expression.

When HEK293 cells stably expressing a CD11d-YFP fusion protein alone are visualized by confocal microscopy CD11d-YFP appears to be confined to the Golgi apparatus. When HEK293 cells stably expressing a CD18-mRFP fusion protein alone are visualized by confocal microscopy CD18-mRFP appears to be confined to the Endoplasmic Reticulum/Golgi complex with no significant surface expression. Transient transfection of CD18-mRFP into CD11d-YFP stably expressing HEK293 cells, or CD11d-YFP into CD18-mRFP stably expressing HEK293 cells, causes relocalization of CD11d-YFP from the Golgi apparatus to the cell surface. CD18-mRFP surface expression was also increased, and colocalized with CD11d-YFP at the cell surface, in those cells that co-expressed both proteins. Flow Cytometry confirmed surface expression of CD11d and CD18 in those cells that co-expressed both proteins.

The C-terminus of CD11d contains the purported CKII phosphorylation site. A GST-CD11d-C-terminal fusion protein was constructed. An *In vitro* kinase assay confirmed the ability of CKII to phosphorylate purified GST-CD11d-C-terminal fusion. However, flow cytometry comparing the surface expression of CD11d to a mutant of CD11d that cannot be phosphorylated by CKII showed no significant effect on the surface expression of CD11d in the presence of CD18. A chimeric fusion protein replacing the C-terminus of CD25 with that of CD11d has been constructed. Recombinant CD25 proteins have been used in the past to study plasma membrane protein trafficking, and we are adapting the system to compare the surface expression of wild type CD25 to the surface expression of chimeric CD25 with its C-terminal domain swapped for the C-terminal of CD11d. Flow cytometry conducted on the constructs have shown the CD25-CD11d C-terminal fusion to have enhanced expression on the cell surface compared to the wild type CD25, suggesting that the CKII site may not be a regulatory region in the C-terminal of CD11d responsible for restricting its movement to the plasma membrane when not heterodimerized with CD18. Future studies will expand the chimeric domain swapping experiments to investigate if regulatory motifs controlling CD11d surface expression exist in the N-terminal and transmembrane domains of CD11d and will also determine if CKII phosphorylates CD11d in tissue culture and *in vivo*. Furthermore, we propose to study the effects of mutating this CKII phosphorylation site on CD11d sub-cellular localization. A further understanding of CD11d biology gained from this study may lead to improved neuroprotective strategies for therapy of secondary SCI.

The role of CnABP in urogenital system development and cancer

ALANA NGUYEN, MELANIE BELAND, YANED GAITAN, MAXIME BOUCHARD

Amongst the most important genes controlling urogenital system development are the Pax transcription factors (Pax2/8). Pax2 homozygous mutants lack kidneys and genital tracts and die within 24 hrs after birth. Pax8 homozygous mutants do not display urogenital defects but Pax8 is found to cooperate with Pax2 in early kidney development. In an effort to isolate genes regulated by Pax2 in the kidney, CnABP was identified as a target gene of Pax2. CnABP is located in the shortest region of overlap at the Wilms' tumor locus 16q24. Further, we showed that CnABP expresses in the condensing mesenchyme of the kidney overlapping with Pax2 and Wt1, a known Wilms' tumor gene. Based on these observations, we hypothesize CnABP is involved urogenital system development and Wilms tumor, a kidney cancer of mesenchymal origin. From screening of 50 Wilms tumors, we identified two mutations in CnABP. To understand the function of CnABP, we expressed CnABP in embryonic kidney cell line HEK293. We showed that CnABP localizes to the membrane through an N-terminus myristoylation signal. Through two independent approaches, we demonstrated that CnABP interacts with Calcineurin A beta. We further showed that CnABP colocalizes with Calcineurin A beta and modulates the phosphatase activities of Calcineurin. Calcineurin is a calcium-dependent phosphatase that signals through the NFAT transcription factor to turn on target genes, some of which are known to be involved in cell cycle regulation. To better assess the role of CnABP *in vivo*, we generated mice from ES-cells containing a genetrap (gt, beta-gal and neomycin fusion) insertion within the CnABP locus. From heterozygous matings, it can be determined that CnABPgt/gt homozygous are not embryonic lethal. However, CnABPgt/gt males are subfertile. Pax2 is necessary for the formation of the nephric ducts, the primordium of the male genital tracts (efferent ducts, epididymis, vas deferens). In addition, Pax2 and Pax8 double heterozygous males are subfertile. We plan to characterize the fertility phenotype of CnABPgt/gt and how this correlates to Pax2 and Pax8 double heterozygous phenotype. To further assess the role of CnABP in any late onset disease or tumor formation, we have generated a cohort of 20 mice of each genotype (CnABP+/, CnABP+/gt, and CnABPgt/gt) that will be followed up to 2 year for any signs of disease.

Key words: CnABP, Pax2, Wilms' tumor, Calcineurin, fertility

Ameliorating benefit assessment procedures for genetically modified organisms

JASON BEHRMANN, BRYN WILLIAMS-JONES

GROUPE DE RECHERCHE EN BIOÉTHIQUE & DÉPARTEMENT DE MÉDECINE SOCIALE ET PRÉVENTIVE, FACULTÉ DE MÉDECINE, UNIVERSITÉ DE MONTRÉAL, MONTRÉAL, QUÉBEC, CANADA

While government regulators have devoted many resources towards assessing the risks of Genetically Modified Organisms (GMOs) in agriculture, they have done little to assess the multifaceted aspects of the benefits society can accrue from such agricultural products. We seek to examine structures in government policy making and suggest places for innovation that will better assess the risks, as well as the benefits, of GMOs so that the commercialization of future products will be based more on factors that aim to provide the greatest good to society, than simply avoiding known or potential risks. For instance, we argue for more input from the general public in the assessment of GMOs, primarily from farmers, who are a key but largely ignored stakeholder in this important public policy issue. Currently, GMOs are assessed by scientific protocols that do not incorporate the fact that farmers often optimize their methods of farming such crops, in ways not predicted by scientists/technologists or the agrochemical companies providing the seed stock. For example, many farmers know to plant crops that are resistant to certain pests in areas that have the greatest level of pest infestation in order to obtain the best control of this problem and greatly reduce their need for additional pesticides. In scientific trials studying pesticide requirements of pest-resistant crops, crops are planted in random fields independent of infestation levels. Thus, scientific trials may underestimate the possible decrease in pesticides needed for these crops in comparison to practical farming procedures. Accurate assessments of pesticide use are essential for the development of relevant and effective health policies that aim to reduce toxic exposure to farmers or the broader build-up of such chemicals in our environment. We aim to clarify additional faults in existing assessment procedures, similar to the example just presented, in order to contribute to the development of more accurate public health policies in relation to the use of GMOs in agriculture in Canada and internationally.

A pediatric genome-wide association study identifies two novel Type 1 diabetes loci

HAKON HAKONARSON^{2,3}, STRUAN F.A. GRANT^{2,3}, JONATHAN P. BRADFIELD², LUC MARCHAND¹, CECILIA E. KIM², JOSEPH T. GLESSNER², ROSEMARIE GRABS¹, TRACY CASALUNOVO², SHAYNE P. TABACK⁴, EDWARD C. FRACKELTON², MARGARET L. LAWSON⁵, LUKE J. ROBINSON², MARIO CAPASSO³, ROBERT SKRABAN², YANG LU¹, ROSETTA M. CHIAVACCI², CHARLES A. STANLEY⁶, SUSAN E. KIRSCH⁷, DIMITRI S. MONOS^{8,9}, MARCELLA DEVOTO^{3,10}, HUI-QI QU¹, CONSTANTIN POLYCHRONAKOS¹

¹DEPARTMENTS OF PEDIATRICS AND HUMAN GENETICS, MCGILL UNIVERSITY, MONTREAL, QUÉBEC, CANADA; ²CENTER FOR APPLIED GENOMICS, ABRAMSON RESEARCH CENTER, THE CHILDREN'S HOSPITAL OF PHILADELPHIA, PHILADELPHIA, PENNSYLVANIA 19104, USA; ³DEPARTMENT OF PEDIATRICS AND DIVISION OF HUMAN GENETICS, THE CHILDREN'S HOSPITAL OF PHILADELPHIA, PHILADELPHIA, PENNSYLVANIA 19104, USA; ⁴DEPARTMENT OF PEDIATRICS AND CHILD HEALTH, UNIVERSITY OF MANITOBA, WINNIPEG, MANITOBA, CANADA; ⁵DIVISION OF ENDOCRINOLOGY, CHILDREN'S HOSPITAL OF EASTERN ONTARIO, UNIVERSITY OF OTTAWA, OTTAWA, ONTARIO, CANADA; ⁶DIVISION OF ENDOCRINOLOGY, THE CHILDREN'S HOSPITAL OF PHILADELPHIA, PHILADELPHIA, PENNSYLVANIA 19104, USA; ⁷MARKHAM-STOUFFVILLE HOSPITAL, MARKHAM, ONTARIO, CANADA; ⁸DEPARTMENT OF PEDIATRICS UNIVERSITY OF PENNSYLVANIA, SCHOOL OF MEDICINE; ⁹DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE, ABRAMSON RESEARCH CENTER, THE CHILDREN'S HOSPITAL OF PHILADELPHIA, PHILADELPHIA, PENNSYLVANIA 19104, USA; ¹⁰CCEB, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PENNSYLVANIA 19104, USA.

Introduction: Type 1 diabetes (T1D) results from autoimmune destruction of pancreatic beta cells. A number of genetic determinants of T1D have already been established through candidate gene studies. These genetic associations with T1D explain little more than half of the genetic risk for T1D. To identify novel genetic factors that confer risk to the pathogenesis of T1D, we performed a genome-wide association (GWA) study. **Methods:** A two-stage design was adapted in this study. (1) In Stage 1, two cohorts of European population were studied, i.e. a case-control study including 499 T1D probands and 1,058 controls, and a family-based study including 483 nuclear families with at least one diabetic child and both parents. We genotyped 550,000 single nucleotide polymorphisms (SNPs) using the Illumina Human Hap550 Genotyping BeadChip. (2) In Stage 2, the 60 most highly significant SNPs from 30 loci were fast-tracked using the SNPlex platform from Sequenom in two other cohorts of T1D families, i.e. 549 nuclear families with 1,333 affected offspring from the Type 1 Diabetes Genetics Consortium (T1DGC), and an additional 390 Canadian families. **Results:** Besides confirming previously identified associations, one locus reached genome-wide significance for T1D association ($P \leq 1.03 \times 10^{-8}$) in Stage 1, and was confirmed in Stage 2. This novel T1D locus involves the gene KIAA0350 on Chr16p13, predicted to be a sugar binding C-type lectin. The gene is expressed in B lymphocytes, dendritic antigen presenting cells and T cells, including NKT-cells, which is in keeping with a function relevant to an immune-mediated disease such as T1D. Of the remaining findings, an additional locus at Chr12q13 which came within an order of magnitude of Bonferroni correction for 550,000 tests was also replicated in Stage 2. The combined analysis of the T1D association with the minor allele of rs1701704 has $p = 2.13 \times 10^{-9}$. **Conclusion:** This study provides proof of principle for the genome-wide association approach and evidence for two novel T1D loci, pointing to previously unknown pathways in the etiology of T1D. **Keywords:** Type 1 diabetes, genome-wide association, single-nucleotide polymorphism.

Biological activity of cross-linked intravenous immunoglobulins (IVIg) on human B cells

DOMINIC PAQUIN PROULX, RÉAL LEMIEUX AND RENÉE BAZIN

DEPARTMENT OF RESEARCH AND DEVELOPMENT, HÉMA-QUÉBEC AND DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY, LAVAL UNIVERSITY, QUÉBEC, CANADA

Therapeutic preparations of intravenous immunoglobulins (IVIg) are derived from the plasma of thousands of blood donors. IVIg were initially used as a supportive therapy for primary or secondary immunodeficiencies. However, IVIg have also been used at very high doses (2g/Kg of body weight) for more than 20 years to treat a significant number of autoimmune or inflammatory diseases, making IVIg the mostly used blood-derived product. The need for high doses of IVIg, combined to the increasing number of diseases treated with IVIg and the limitation of plasma available for fractionation could lead to product shortages in a near future.

One example of an autoimmune disease treated with IVIg is the immune thrombocytopenic purpura (ITP). ITP patients produce autoantibodies against their own platelets, leading to their destruction and resulting in bleeding problems. In ITP patients treated with IVIg, short and long term effects are observed. Short term effects include a rapid inhibition of platelet phagocytosis while long term effects include a reduction of autoantibody titers in the patient's serum. We recently showed that small size immune complexes prepared by cross-linking IVIg with a mouse monoclonal anti-human IgG (C5-1) were about 10 times more efficient than IVIg to prevent platelet destruction in a mouse model of ITP, suggesting that the same therapeutic efficacy could be achieved with less IVIg using this cross-linking strategy.

In this work, we studied whether the long term effects of IVIg treatment observed in ITP patients (reduction of autoantibody titers) could also be obtained with cross-linked IVIg. We have previously reported that IVIg induced the differentiation of human B cells using an in vitro culture system. The increase in differentiation was characterized by a decrease in proliferation of about 50% and an increase in IgG secretion. We show here that cross-linked IVIg are about 30 times more potent than IVIg in inducing human B cell differentiation. Furthermore, our results show that this effect is not dependent on the classical receptor for immunoglobulins expressed on B cells (FcγRIIb) and suggest that it may involve a new class of immunoglobulin receptors (FcRL).

Apoptosis in epithelial fetal lung cells exposed to stretching as a result of positive ventilation

NATHALIE RICHARD, IRENE TSEU AND DR. MARTIN POST

DEPARTMENT OF LUNG BIOLOGY, THE HOSPITAL FOR SICK CHILDREN, 555 UNIVERSITY AVENUE, TORONTO, CANADA

The use of positive mechanical ventilation and oxygen in pre-term infants who have respiratory distress syndrome (RDS) can lead to Bronchopulmonary Dysplasia (BDP). Lungs that develop BDP are at risk of developing inflammation, alveolar arrest, pulmonary hypertension, and fibroblast hyperplasia. In addition, high levels of apoptosis have been found in the lungs of patients who suffer from RDS.

Apoptosis is controlled cell death that is characterized by cell condensation, nuclear fragmentation and changes to the morphology of the cell membrane. Unlike cell necrosis which results in the release of all the components of the cell, apoptosis culminates in cell death without inflammation. Two caspase-dependent pathways are implicated in apoptosis, namely the intrinsic and extrinsic pathways. The intrinsic pathway is characterized by the release of cytochrome C and subsequent caspase 9 activation while the extrinsic pathway is characterized by the activation of the Fas (CD95/APO1) receptor and caspase 8 cleavage. Both pathways lead to the production of cleaved caspase 3, the effector caspase. *We hypothesize that both pathways are involved in the overdistension-induced apoptosis of lung epithelial cells.* To test the hypothesis, freshly isolated fetal day 19 rat lung epithelial cells were seeded onto Bioflex culture plates (Flexercell International) and subjected to a continuous 20% stretch for 6 hours. Afterwards, cells were lysed and samples were analyzed by Western blotting. Our results indicate that un-stretched samples have lower levels of cytochrome C and cleaved caspase-3 than stretched samples. Future experiments will include Western Blots using caspase-8, Fas and Apaf-1 antibodies to confirm the presence of these pathways.

Keywords: apoptosis, caspase-3, cytochrome C, stretch

Idiopathic toe walking: a marker for developmental delay

M RADINA*, MJ PENNER*, RG SMITH, D SAMDUP

FACULTY OF HEALTH SCIENCES, QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, CANADA

*These authors contributed equally to this work.

Background: Idiopathic Toe Walking (ITW) is a diagnosis of exclusion in children who display this gait pattern without associated neuromuscular disease. Previous smaller studies have found an association of ITW with autism and various developmental delays, specifically in speech, language and learning. **Objectives:** The aim of this study is to re-examine the association between ITW and developmental delay by conducting a larger trial of children seen in an orthopaedic clinic. **Design/Method:** Parents or guardians were contacted by telephone and were asked to complete a survey, which was designed by two developmental paediatricians. Results were analysed by a separate data analyst at Kingston General Hospital. **Results:** Forty three parents completed the survey. The mean age of children was 8.0±2.4 years (range of 3.5 to 12.3), and 58% of the sample was male. Diagnosed developmental delays included speech/language delay in 15 (35%) children, motor delay in 5 (12%) children, and learning disabilities in 8 (19%) children. ADHD and autism were diagnosed in 9 (21%) and 5 (12%) children, respectively. In those without a diagnosis of autism, an additional 29% displayed one or more red flags of behaviour known to be exhibited by children with autism. **Conclusion:** These results support previous research and suggest that developmental delay, autism and ADHD have a higher prevalence in children with ITW. Hence, a diagnosis of ITW should be considered a red flag for potential neurodevelopmental issues and warrants developmental screening.

Keywords: idiopathic toe walking, developmental delay, autism, ADHD

Optimization of human serum albumin nanocapsules for drug delivery applications

SAFAA SEBAK, MARYAM MIRZAEI, MEENAKSHI MALHOTRA, ARUN KULAMARVA AND SATYA PRAKASH

BIOMEDICAL TECHNOLOGY AND CELL THERAPY RESEARCH LABORATORY, DEPARTMENT OF BIOMEDICAL ENGINEERING, FACULTY OF MEDICINE, MCGILL UNIVERSITY, MONTREAL, QUEBEC, CANADA

Introduction: Despite impressive results available chemotherapeutic drugs have severe symptoms associated with them including gastrointestinal toxicity, susceptibility to the development of drug resistance, poor bioavailability that results in a need for prolonged intravenous infusions, and the use of toxic solubility agents. Thus, new methods of drug delivery are being explored to minimize harmful side-effects through the use of nano-scaled drug delivery systems. The objective of this study is to design albumin nanocapsules of controllable size and investigate their properties for the optimal, safe and effective deliverance of anti-cancer drugs. **Methods:** Novel albumin nanocapsules were designed, prepared and characterized in-vitro. 50-200mg of human serum albumin (HSA) was dissolved in 10mM NaCl. The pH was adjusted to values 7-11 by adding NaOH. Ethanol was added dropwise at a defined rate (ml/min) using a peristaltic pump to form uniform nanocapsules. The nanocapsules were stabilized by crosslinking with 8% and 25% grade glutaraldehyde. Particles were purified by a 4-fold centrifugation (50,000 rpm, 15min) to remove free albumin and excess glutaraldehyde. Centrifuged particles were resuspended in PBS, and lyophilized. The nanocapsules were studied under scanning electron microscope (SEM) and particle size, stability, and zeta potential were analyzed and compared to determine optimal conditions in order to achieve a colloidal system with well-defined physicochemical characteristics. **Results:** The albumin nanocapsules were prepared varying the HSA concentration, pH, and crosslinker in order to optimize the preparation procedure with respect to a defined particle size and distribution. The optimal preparation conditions resulted in controllable particle size between 150-500nm. The first study was the effect of HSA concentration on particle size as the HSA concentration increased, the particle diameter decreased. The pH value of the HSA prior to ethanol addition strongly influenced the resulting particle size. At pH>7 the particle size was significantly reduced with increasing pH value. At pH 8, the particles were stable with the size increasing by less than 20nm. No influence of crosslinking conditions on the resulting particle size was observed. Considering the results on the influence of different parameters under evaluation, a standard protocol for the preparation of HSA nanocapsules was established. **Discussion:** These findings reveal a correlation between preparation conditions of nanocapsules and physicochemical characteristics. Literature suggests that this method of preparation of nanocapsules has potential resulting in nanocapsules with the required pharmacokinetics to selectively target cancerous cells, improve drug stability, and increase bioavailability. Surface modification procedures aiming at specific drug binding and targeting have yet to be investigated.

Mitochondrial targeting and folding functions of chaperones

S. TZANKOV, M. K. BHANGOO, A.C.Y. FAN AND J.C. YOUNG

DEPARTMENT OF BIOCHEMISTRY, MCGILL UNIVERSITY, MONTREAL, QUEBEC, CANADA

Mitochondrial preproteins that are imported via the Tom70 receptor are complexed with the cytosolic chaperones Hsp90 and Hsc70 before targeting to the mitochondrial outer membrane. Dissection of the pathway with Hsp90 inhibitors found that the chaperone helped to maintain preproteins in the cytosol before import, to target preproteins onto the Tom70 receptor, and to promote translocation across the mitochondrial outer membrane. To identify other proteins involved, a purified mitochondrial preprotein was reconstituted with chaperones in reticulocyte lysate, and bound proteins were identified by mass spectrometry. In addition to Hsc70 and Hsp90, a specific subset of co-chaperones was found, but no mitochondria-specific targeting factors. Interestingly, three different Hsp40-related J-domain proteins were identified: DJA1, DJA2 and DJA4. The DJAs bound preproteins to different extents through their C-terminal regions. DJA dominant negative mutants lacking the N-terminal J-domains impaired mitochondrial import. The DJAs also showed significant differences in activation of the Hsc70 ATPase and Hsc70-dependent protein refolding. In HeLa cells, the DJAs increased the activity of newly synthesized luciferase and mitochondrial accumulation of a preprotein, although to different extents. No single DJA was superior to the others in all aspects, but each had a profile of partial specialization. We suggest that multiple co-chaperones with similar yet partially specialized properties cooperate in optimal chaperone-preprotein complexes.

Keywords: protein folding, mitochondrial import, chaperones, Hsp70, Hsp90

A novel pathway of cadherin, Rac1/Cdc42 and Stat3 interaction

ROZANNE ARULANANDAM¹, JUN CAO¹, ADINA VULTUR¹, JONATHAN DEGEER¹, LIONEL LARUE², HÉLÈNE FERACCI³ AND LEDA RAPTIS¹.

¹DEPARTMENT OF PATHOLOGY, QUEENS' UNIVERSITY, KINGSTON, ONTARIO, CANADA. ²INSTITUT CURIE, PARIS, FRANCE.

³CENTRE DE RECHERCHE PAUL PASCAL, BORDEAUX, FRANCE

Cellular interactions with neighboring cells profoundly influence a variety of signalling events including those involved in mitogenesis, survival and differentiation. Unlike cultured cells, cells in a tumor have extensive opportunities for adhesion to their neighbors in a three-dimensional structure, therefore in the study of signal transduction it is important to take into account the effect of neighboring cells. The Signal Transducer and Activator of Transcription-3 (Stat3) has emerged as an important signaling molecule with a role in the etiology of many cancers, such as breast cancer, and is required for transformation by a number of oncogenes.

Our lab has demonstrated that cell-to-cell adhesion, as observed in confluent cultured cells, can lead to a dramatic increase in Stat3 activity, which peaks at 2-4 days post-confluence. Most importantly, this Stat3 activation required calcium but was resistant to inhibition of a number of tyrosine kinases, often activated in a variety of cancers. Downregulation of Stat3 induced apoptosis, which was most prominent in post-confluent cell cultures, indicating that Stat3 plays a central role in regulating cell survival (Oncogene 2004). Cadherins have recently emerged as a group of cell-cell adhesion molecules involved in the regulation of signalling events, as well as the maintenance of tissue architecture. It has been shown that cadherin engagement can result in the rapid activation of the Rac1 and Cdc42, Rho family GTPases. Furthermore, expression of activated forms of the Rho GTPases can lead to Stat3 phosphorylation and activation. In this report we demonstrate that E-cadherin engagement can activate Stat3. Stat3 activation is preceded by a dramatic increase in the activity as well as the levels of Rac and Cdc42 and is followed by potent survival signalling. Moreover, downregulation of Rac and Cdc42 through expression of dominant-negative mutants or pharmacological inhibitors caused a dramatic reduction in Stat3-tyr705 levels, indicating that their activation may be part of the pathway whereby E-cadherin engagement leads to Stat3 activation and survival.

Keywords: Stat3, cadherin, Rho GTPases, cell-cell adhesion, confluence

ADAM12 effects on Dupuytren's Disease cell morphology and cytoplasmic beta catenin accumulation require Type I IGF receptor tyrosine kinase activity

LINDA VI1,2,7, BING SIANG GAN1,2,3,4,5,7, DAVID O'GORMAN1,2,3,6,7

CELL & MOLECULAR BIOLOGY LABORATORY, HAND & UPPER LIMB CENTRE, ST. JOSEPH'S HEALTH CARE1, LAWSON HEALTH RESEARCH INSTITUTE2, DEPARTMENTS OF SURGERY3, PHYSIOLOGY AND PHARMACOLOGY4, MEDICAL BIOPHYSICS5, BIOCHEMISTRY6, UNIVERSITY OF WESTERN ONTARIO, LONDON, ONTARIO, CANADA7

Background: Dupuytren's disease (DD) is a debilitating fibroproliferative disease of the palmar fascia in the hand that results in the formation of a collagenous disease cord and permanent contraction of affected fingers. Our laboratory has previously documented increased levels of cytoplasmic beta-catenin, a signalling molecule involved in cell proliferation, in DD. The signalling pathway regulating beta-catenin accumulation in DD is not known. Affymetrix microarray analysis of surgically resected DD tissue performed in our laboratory indicates that A Disintegrin And Metalloprotease (ADAM) 12 is highly expressed in this fibrosis. This extracellular matrix-associated protease has been shown to increase Type I Insulin-like Growth Factor Receptor (IGFRI) tyrosine kinase activity, promote adherens junction disruption, and increase cytoplasmic levels of beta-catenin in other systems. The purpose of this study was to determine if ADAM12 affects beta-catenin accumulation in DD and, if so, whether this was dependent on IGFRI tyrosine kinase activity. **Methods:** Cells derived from DD patients were cultured on Type-1 collagen and treated with exogenous ADAM12 / vehicle in the presence or absence of NVP-ADW742-NX-7, a specific inhibitor of IGFRI tyrosine kinase activity. To more closely replicate the in vivo disease environment, a collagen co-culture method was employed. Briefly, primary DD cells were cultured on insert wells coated with collagen containing ADAM12 / vehicle, in co-culture with normal PF or DD cells cultured on untreated collagen. Immunofluorescence microscopy and Western blotting were used to analyze cell morphology and beta-catenin levels. **Results:** The addition of exogenous ADAM12 to DD cells, but not normal PF cells, resulted in marked changes in cellular morphology including condensed actin stress fibres and cytoplasmic beta-catenin accumulation. These effects of ADAM12 on DD cells were strongly inhibited by NVP-ADW742-NX-7, an IGFRI-specific tyrosine kinase inhibitor. Further, DD cells grown on collagen in co-culture with DD cells grown on collagen containing ADAM12 displayed increased beta-catenin levels relative to controls. **Conclusions:** This study demonstrates for the first time that inhibition of IGFRI tyrosine kinase activity markedly inhibits changes in cell morphology and that beta-catenin accumulation is induced by ADAM12, consistent with IGFRI signalling being an essential component of beta-catenin accumulation in DD.

Keywords: Dupuytren's Disease, ADAM12, IGF, tyrosine kinase

Caspase substrates screening by diagonal gel approach and study on caspase-1 substrates on glycolytic pathway

W. SHAO¹ AND M. SALEH²

¹DEPARTMENT OF BIOCHEMISTRY, MCGILL UNIVERSITY, MONTREAL, QUEBEC, CANADA. ²DEPARTMENT OF MICROBIOLOGY & IMMUNOLOGY, MCGILL UNIVERSITY AND CRITICAL CARE DIVISION, ROYAL VICTORIA HOSPITAL, MONTREAL, QUEBEC, CANADA

Apoptosis is executed by caspase-mediated cleavage of various proteins. Elucidating the consequence of substrate cleavage provides us insight into cell death and other biological processes. In this study, we applied the diagonal gel approach, a proteomic strategy, to screen for caspase-1 and -3 substrates. Our results showed significant overlap between caspase-1 and -3 substrates obtained by the diagonal gel approach. Substrates for both caspase-1 and caspase-3 are implicated in common cellular functions, such as maintenance of the cytoskeleton, chaperons, translation, glycolysis, bioenergetics, signaling and trafficking. An important finding is that many glycolysis enzymes are targeted by caspase-1 in the diagonal gel approach. Cleavage of these glycolysis enzymes was confirmed by cleaving in vitro transcribed and translated substrates with recombinant caspase-1. Point mutation of GAPDH blocks its cleavage by caspase-1. This provides a direct link between apoptosis and glycolysis.

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ERRATUM

In the last issue of the MJM, Volume 10, Number 2, Victoria Au's abstract should have been included among scientific abstracts from the CSLSR conference. We include her abstract below. The MJM apologizes for any inconvenience this error may have caused.

Characterizing the functional domains of baculovirus Late Expression Factor 3 (LEF-3)

VICTORIA AU AND DR. ERIC CARSTENS
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY, QUEEN'S UNIVERSITY, KINGSTON,
ONTARIO, CANADA

The *Baculoviridae* is a family of arthropod-specific viruses found in over 600 insect species. Baculovirus species have very specific host ranges and are unable to replicate in mammalian cells, making them ideal for use as biopesticides and vectors for gene therapy. AcMNPV is the best-studied member of the *Baculoviridae* family and most of the genes identified in this virus serve as a basis for comparison to other baculoviruses. Six proteins are essential for AcMNPV DNA replication: LEF-1, LEF-2, LEF-3, DNA Polymerase, P143, and IE-1. LEF-3 (407 aa, 45 kDa) is a single-stranded DNA binding protein that functions as a homotrimer. LEF-3 also transports P143, a helicase, to the nucleus. We predict that LEF-3 has several functional domains responsible for: ssDNA binding, interaction with P143, and interaction with host importin proteins for nuclear localization. Site directed mutagenesis revealed that the N terminal region (amino acids 5 to 56) are responsible for LEF-3 nuclear localization, while a region including 125 amino acids at the N-terminus is required for interaction with P143. To identify amino acids within the 5 to 56 region essential for nuclear transport, conserved amino acids were targeted for mutagenesis. The intracellular localization of mutated proteins expressed by plasmid-transfected cells was examined using fluorescence microscopy and biochemical fractionation. A similar approach will be used to identify the regions responsible for P143 interaction and ssDNA binding. Although the first fifty amino acids of AcMNPV LEF-3 contain characteristics similar to classic NLSs, the results of mutating 14 conserved amino acids or from deleting various regions within the 5 to 56 region of LEF-3 did not demonstrate any clear effect on nuclear localization. Therefore, the essential components of the domain remain to be determined and the transport of LEF-3 into the nucleus may be mediated by a novel NLS.