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THE ROLE OF NATURAL KILLER CELLS IN CHIMERISM INDUCTION IN THE NON-OBESE DIABETIC MOUSE

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Background: Islet transplantation has been a promising treatment option for patients with diabetes mellitus; however, a major obstacle is the recipients' immune response against the donor tissue and autoantigens. Establishing chimerism by bone marrow transplantation (BMT) can lead to immunological tolerance in the Non-Obese Diabetic (NOD) mouse model of human type 1 diabetes. However, chimerism induction in the NOD mouse is a difficult process and it is hypothesized that Natural Killer (NK) cells may be a barrier to chimerism induction. NK cell tolerance to transplanted BM may lead to stable mixed chimerism, and therefore, the role of NK cells in chimerism induction needs to be defined.

Methods: In order to determine the role of NK cells in the NOD mouse resistance to chimerism, we have designed a series of experiments to observe the efficiency of allogeneic bone marrow engraftment and the establishment of chimerism in the absence of NK cells. NOD mouse NK cells were depleted, knocked-out, or rendered non-functional, and these NOD mice were subsequently made chimeric through a C3H mouse (fully allogeneic) bone marrow transplant.

Results: NK cell depletion in NOD mice significantly enhances hematopoietic cell chimerism from fully allogeneic C3H bone marrow.

Conclusion: Preliminary data suggests that hematopoietic chimerism in NOD mice with donor C3H bone marrow cells is accomplished with greater efficiency after the depletion of recipient (NOD) NK cells. Further experiments are planned to determine the targets of NOD NK cell killing and tolerance of the chimeric mice to donor transplants.

FACTORS TO INFLUENCE LENGTH OF STAY FOR IN-PATIENT MALIGNANT HEMATOLOGY SERVICE: MULTILEVEL MODELING

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Background: Predicting the length of stay for in-patients is useful to estimating costs and allocating resources for patient care. There is no published model in medical oncology and including hematology patients.

Methods: Data was obtained from the Discharge Abstract Database (DAD) maintained by Canadian Institute for Health Information (CIHI) for the year 2002-2005. Admission to hematology service at Henderson General Hospital in Hamilton, which is tertiary referral center was used to minimize variability due to site and caregivers.

Potential factors were modelled to be nested among individual patients (longitudinal multi-level modeling).

We designed the model to make as simple as possible and maximize its clinical use. We only included factors available on admission.

Factors entered in the model: Age, Age squared, Port of entry to the hospital (direct vs. ER vs. other), County of residence (Hamilton vs non-Hamilton), Gender, Day of the admission (weekday vs. weekend), Diagnosis and Number of hospital admissions.

Results: 713 patients with 1739 admissions.. factors with non-significant B(slope) value were omitted from the model. Age is centered around the mean. The final model is:

$$LOS_{j(est)} = 3.9658 + u_{00} + (.2068 + u_{1j})(Age - 65) + (4.508 + u_{2j})(\text{number of visits}) + r_j$$

In another ward for each increment by 5 years above the average the LOS increases by 1 day and for each subsequent admission the LOS increase by 4.5 days.. The Pseudo-R squared is ~50%

Conclusion: We developed a simple model which allows the clinician to anticipate patients who are at risk of prolonged LOS and may allow for institution of earlier interventions in an attempt to shorten LOS.

DEVELOPMENT OF A STEM CELL DELIVERY SYSTEM TO TREAT RETINAL DEGENERATIVE DISEASES

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Background/Purpose: Retinal disease leads to permanent vision loss for which there is no regenerative treatment. The therapeutic potential of adult retinal stem cells (RSCs) avoids ethical concerns surrounding transplantation of embryonic/fetal tissue. Subretinal transplantation is limited by poor cell survival, distribution and integration into host tissue. A stem cell delivery system was developed using the minimally-invasive, injectable and biodegradable properties of a blend of hyaluronan (HA) and methylcellulose (MC) – HAMC.

Methods: Polymer hydrogels (agarose, collagen, chitosan/glycerol-phosphate, and HAMC) were screened *in vitro* for desired physical and biological properties. Cell growth and survival was analyzed *in vitro* by RSC sphere diameter and live-dead assays. HAMC was pursued in adult mouse subretinal transplantation studies to investigate biodegradability and as a delivery vehicle using GFP+ RSC progeny.

Results: Several hydrogels were eliminated: chitosan/glycerol-phosphate based on long gelation time, collagen because of retarded cell growth, and agarose based on cell spreading/differentiation. A blend of 0.5/0.5 wt% HA/MC supported *in vitro* stem cell growth and survival. HAMC maintained 3D cellular distribution and prevented aggregation. The blend was shown to degrade over 7 days *in vivo*. Transplanted RSC progeny integrated into the retinal pigment epithelium layer. Compared to saline, when delivered in HAMC cells were more contiguously distributed over larger areas of retina, eliminating patch-like integration and cellular aggregation.

Conclusion: HAMC is a promising vehicle for cellular delivery to the degenerating retina, overcoming barriers to tissue integration such as cellular aggregation and non-contiguous distribution.

REDUCTIONS IN QUADRICEPS STRENGTH AND POWER ACROSS A CLINICAL SPECTRUM OF KNEE OSTEOARTHRITIS

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Purpose: To determine the effect of clinical disease severity on quadriceps muscle strength, velocity and power in patients with knee osteoarthritis (OA).

Methods: Thirty patients with symptomatic knee OA were stratified into tertiles representing mild, moderate and severe OA based on responses to the Western Ontario and McMaster Osteoarthritis Index (WOMAC). Isometric strength, isotonic power (measured at 10, 20, 30, 40 and 50% MVC) and maximal velocity (V_{MAX}) were assessed in the quadriceps unilaterally. Multiple simple linear regression models were used to determine whether strength or power parameters were most predictive of variance in self-reported function.

Results: Quadriceps muscle strength was reduced by 52.1% when comparing mild and severe groups. Velocity was significantly lower in the severe versus mild groups at V_{MAX} , 10 and 20% MVC and between moderate and severe groups at 10 and 20% MVC. Muscle power was significantly lower at 20, 30, 40, 50% MVC when comparing mild and severe groups. Power at 20% MVC explained the most variance of any parameter in WOMAC function subscale (34%).

Conclusion: Quadriceps muscle strength, velocity and power are reduced across a clinical spectrum of knee OA. Power, especially at high contraction velocities is an important predictor of subjective level of function. Interventions should target those with mild and moderate knee OA in order to prevent the strength and power deficits experienced by those with severe knee OA.

ADHERENCE TO ANTIRETROVIRAL THERAPY: SOCIAL IDENTITY, MARGINALITY, AND THREATS TO AGENCY

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Background: Adherence to antiretroviral therapy (ART) is a pressing concern for women living with HIV/AIDS in Vancouver's inner city. Although there have been vast improvements in the simplicity and tolerability of medications and a wide variety of health and ancillary services available, good clinical outcomes remain elusive.

Methods: Our analysis of ART adherence draws on an examination of health behaviour literature, along with 15 months of qualitative research in Vancouver's downtown eastside. Participant observation was conducted in a variety of clinical settings, and open-ended interviews were carried out with physicians (n=8), nurses (n=9), social and outreach workers (n=2), health administrators (n=2), and patients (n=7). Our interpretive analysis was triangulated through on-going consultation and follow-up with participants.

Results: Adherence is commonly modeled as rational action, and methodological individualism endorsed as adequate for understanding challenges with ART. Our qualitative investigation reveals a more complex set of interpersonal and structural dynamics: women's treatment on the basis of their social identities as drug users strongly impacted health care engagement, as did shared social meanings attached to medications and illness. In the inner city context, all-cause mortality is high—HIV is not, for women in *this* project, a manageable, chronic disease.

Conclusions: This analysis demonstrates how conventional approaches to adherence fail to capture social and interpersonal dynamics of medical care. Our understanding of adherence emphasizes social interests, institutional authorities, relations of power, and strategies of social control, which are exerted on, resisted, and internalized by women attempting to negotiate care.

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DE NOVO DESIGN OF ANTIMICROBIAL PEPTIDES FROM MAMMALIAN HISTONES

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Background: Histones have been observed to exhibit antimicrobial activity [J. Exp. Med. 108:925] and to play a crucial role in the ability of neutrophil extracellular traps to inhibit bacteria [Science 303:1532]. It is hypothesized that by synthesizing specific regions of the mammalian histone genome with properties similar to other known antimicrobial peptides, new polypeptides with antimicrobial activity may be found.

Methods: Five mammalian histone sequences were synthesized based on the amphipathicity and cationic charge [Biochemistry 31:12688]. Biomimetic large unilamellar vesicles representative of mammalian membranes and bacterial membranes were composed of mixtures of different lipid species and extracts. Using biophysical techniques the bactericidal activity and the mechanism of interaction of the peptides were established.

Results: Isothermal Titration Calorimetry (ITC) showed no binding for the peptides with mammalian vesicles. However, peptides H2A1 and H3 bound to bacterial membranes and *E. coli* extracts. Differential scanning calorimetry mirrored ITC results with large perturbations in the phase transitions for the two peptides with bacterial membranes. Dye leakage and circular dichroism data correlated well, indicating a helical structural transition with leakage from the bacterial membranes for the same two peptides. Live-dead assays and minimal inhibitory concentration showed a significant bactericidal effect with peptides H2A1 and H3.

Conclusions: Two antimicrobial peptides H2A1 and H3 were successfully derived from mammalian histones, demonstrating specificity for bacterial vesicles accompanied with membrane disruption, and a helical conformation. This result provides insight into a potential antibacterial role for histones in the immune system and points to potentially new pharmaceuticals for treating bacterial infections.

PRIOR CHEMOTHERAPY IMPAIRS ONCOLYTIC VIRUS-INDUCED ANTI-LEUKEMIA IMMUNITY

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Background/Purpose: Malignant cells have multiple mutations, resulting in aberrant signalling pathways, which oncolytic viruses (OVs) exploit for replication. OVs infect and kill many malignant cell lines including murine leukemia L1210. Despite OV success in treating many types of solid tumours, OVs are less effective in established leukemia or lymphoma. However leukemic cells infected with an OV ex-vivo, can induce specific anti-leukemic immunity. We wished to examine the effect of prior chemotherapy on the immune response.

Methods: L1210 cells were infected with an engineered OV (d51-VSV) which was then used to vaccinate, via the tail vein, naïve or cyclophosphamide (50mg/kg) treated DBA mice (5/group) once weekly x 3. This was followed by lethal leukemic challenge.

Results: Leukemia free survival was 100%, 20% and 0% in the vaccinated, cyclophosphamide-vaccinated and control groups respectively. Similar results have been repeated with an alternate engineered OV with consistently better survival rates in the chemotherapy cohorts, using a variety of drug combinations and intensities.

Conclusion: OV tumour lysates provide consistently robust protection from lethal leukemic challenge. The effect of previous chemotherapy must be overcome to translate this into the treatment of MRD after remission induction.

Reference: Bell JC. Oncolytic viruses: what's next? *Curr Cancer Drug Targets* 2007; 7: 127–131.

FETAL STAGE HEMATOPOIETIC STEM AND PROGENITOR CELLS DIFFER FROM THEIR ADULT COUNTERPARTS BY ELEVATED HIGH-MOBILITY GROUP A2 (HMGA2) EXPRESSION AND CORRESPONDINGLY LOW EXPRESSION OF ITS REGULATORY COUNTERPART LET-7B

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Background/Purpose: Umbilical cord blood (CB) is a rich source of blood-forming hematopoietic stem cells (HSCs) and is therefore increasingly utilized for stem cell transplantation; however, adult patients cannot currently exploit this source of HSCs due to the prohibitively low number of active cells in each sample. The purpose of this study is to identify genes that are responsible for the faster in vivo repopulating activity that we have shown to characterize transplanted fetal as compared to adult HSCs as a potential first step in identifying new ways for enhancing the in vivo activity of CB HSCs to become useful for adult patients.

Methods: Nearly pure populations of viable HSCs were isolated from mouse E14.5 fetal liver (FL) and adult (10-12 week) bone marrow (BM) using novel combinations of cell-surface markers and fluorescence activated cell sorting. The expression levels of selected transcripts in these cell isolates were then measured using quantitative real-time PCR.

Results: Of 19 candidate genes selected based on previous comparative global transcript analyses, we found that 2 transcriptional regulators (*Smarcc1* and *Hmga2*) are differentially expressed between FL and BM HSCs; however, only *Hmga2* maintains its differential expression after in vitro stimulation. We also found that *let-7b*, a known microRNA negative regulator of *Hmga2*, is higher in HSCs from adult BM as compared to FL HSCs.

Conclusion: *Hmga2* is expressed at a higher level in fetal compared to adult HSCs and this decrease in expression in HSCs correlates with a corresponding developmental increase in *let-7* micro-RNA expression, particularly *let-7b*.

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FUNCTIONAL LIMITATIONS AND SERVICE NEEDS OF ADULTS WITH 22Q11.2 DELETION SYNDROME

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Background/Purpose: There is little known about long term outcome, functioning, and service needs of adults with emerging genetic syndromes. 22q11.2 deletion syndrome (22q11DS) is the most common microdeletion syndrome known, and is characterized by multiple developmental and later onset features, including learning difficulties, congenital heart disease (CHD), and schizophrenia (SZ). We investigated the impact of these major features on overall functioning in adults with 22q11DS.

Methods: We studied 85 adults with 22q11DS, including n=39 with SZ. The Vineland Adaptive Behaviour Scale (VABS) was completed by caregiver interview. Multivariate regression analyses used covariates age, sex, CHD status, SZ status, and IQ. A mail survey concerning medical and social service use by these adults was also sent to all caregivers.

Results: Considerable functional impairment was observed across all VABS domains. Functioning in the daily living skills (DLS) area was a relative strength. There was significantly greater functional impairment in subjects with SZ compared with those with no history of psychosis across most domains (communication, $p=0.0610$; DLS, $p=0.0001$; socializing, $p=0.0034$; total composite score, $p=0.0003$). Presence of severe CHD had no significant effects. Survey results are pending.

Conclusion: Vocational and other services must be aware of widespread functional limitations in adults with 22q11DS, and should take advantage of relative strengths in domestic and community interaction skills. The additional diagnosis of SZ brings a new set of issues to be faced for the individuals with 22q11DS, service planners, families, and other providers of care. Syndrome-specific education for health professionals and service providers may help optimize functioning.

P63 ANTAGONIZES P53 TO PROMOTE THE SURVIVAL OF MOUSE NEURAL PRECURSOR CELLS DURING EMBRYONIC DEVELOPMENT

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Background: The cerebral cortex develops from a pool of neural precursors that sequentially generates neurons and then glial cells. From embryonic day 12 (E12) to E18 some cells undergo apoptosis as part of a cell death program that helps to restrict the number of precursor cells to determine brain size. The molecular mechanisms that regulate survival of neural precursors are still relatively ill-defined. Increasing evidence suggests that the p53 tumor suppressor and its related family members p63 and p73 play important roles in stem cells, and we have previously shown that p63 is expressed in developing neural precursors of the embryonic cortex.

Method: We asked if p63 plays any role in neural precursor development, focusing upon the embryonic cortex using *in vitro* and *in vivo* assays.

Results: Knockdown of p63 either in culture or *in vivo* causes apoptosis of cortical precursors and newly born cortical neurons. Cortical precursor apoptosis is the consequence of deregulated p53 activity, since apoptosis induced by loss of p63 is rescued by coincident silencing of p53. When p63 levels in cortical precursor cells are ablated, proliferation is unaltered suggesting that the primary effect of p63 knockdown is to induce apoptosis. We show that $\Delta Np73$ does not alter survival of cortical precursor cells, but that it collaborates with $\Delta Np63$ to ensure the survival of newly-born cortical neurons.

Conclusions: The balance of $\Delta Np63$ versus p53 determines the life versus death of embryonic cortical precursors, a role that may extend to other populations of developing and/or adult stem cells.

PREVENTION OF GENOMIC INSTABILITY BY CYCLIN-DEPENDENT KINASE 5

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Background/Purpose: Cyclin-dependent kinase 5 (Cdk5) is a small protein serine-threonine kinase that regulates the activity of ATM and p53 (1, 2), critical proteins for proper DNA repair and apoptosis. Given the important role of these proteins in preventing genomic instability, we hypothesize that deregulation of Cdk5 activity may be linked to aberrant DNA break repair function, thereby causing genomic instability and tumorigenesis.

Methods: Mouse embryonic fibroblast (MEF) cells obtained from wild type and Cdk5^{-/-} mice were cultured under high oxidative stress conditions (20% O₂). The baseline level of DNA damage in individual cells was assessed by a Comet Assay kit (Trevigen). The development of aneuploidy in these cells was measured through Fluorescence Activated Cell Sorter (FACS) analysis and through manual chromosome counts of mitotic cell spreads.

Results: Cdk5^{-/-} MEFs had a significantly greater comet length than Cdk5^{+/+} MEFs (191.9 ± 10.48 vs 131.2 ± 5.978 arbitrary units, respectively, P = 0.0001; n ≥ 70 cells). At passage 4, a chromosome count of mitosis-arrested cells showed a significantly higher percentage of Cdk5^{-/-} MEFs with aneuploid chromosome numbers compared to Cdk5^{+/+} MEFs (82.4% vs 57.8%, respectively, P = 0.005, n ≥ 90 cells). By passage 10, FACS analysis confirmed a significantly higher population of aneuploid cells in Cdk5^{-/-} MEFs compared to Cdk5^{+/+} MEFs (48.7% vs 33.7%, respectively, P < 0.05; n ≥ 10 000 cells).

Conclusion: Cdk5 appears to play an important role in preventing DNA damage and aneuploidy in MEF cells.

References: (1) Zhang, J., et al. J Neurochem, 81-2: 307. (2) Tian, B., et al. Nat Cell Biol, 11-2: 211.

CARDIOPROTECTIVE EFFECTS OF CYCLOSPORINE IN A NEWBORN PIGLET MODEL OF ASPHYXIA: A DOSE-RESPONSE STUDY

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Introduction: Myocardial depression following asphyxia of the newborn is a significant cause of mortality. Cyclosporine reduces myocardial damage in adult patients undergoing percutaneous coronary intervention for myocardial infarction. However, the potential cardioprotective effects of cyclosporine in neonates have not yet been studied. We hypothesize that cyclosporine in asphyxiated newborn piglets will improve cardiac function, systemic hemodynamics and oxygen metabolism.

Methods: Thirty-six piglets (1-4 days-old) were instrumented for continuous monitoring of cardiac output and systemic arterial pressures. After stabilization, normocapnic alveolar hypoxia (10–15% oxygen) was instituted for 2h followed by reoxygenation with 100% oxygen for 0.5h, then 21% for 3.5h. Piglets were block randomized to receive one of three cyclosporine intravenous boluses (2.5, 10 or 25 mg/kg) or placebo (normal saline, control) after 5 minutes of 100% reoxygenation (n=8/group). Blood samples were collected for analysis of blood gases and plasma troponin. Statistical analysis performed using ANOVA.

Results: All piglets demonstrated cardiogenic shock (cardiac output 45% of baseline), hypotension (systemic arterial pressure 30mmHg) and acidosis (pH=7.04) at the end of 2h of hypoxia. Cyclosporine treatment at reoxygenation caused dose-related improvements in cardiac output and oxygen delivery compared to controls (both P<0.05). Cyclosporine at 10 mg/kg significantly improved stroke volume compared to controls, demonstrating preservation of cardiac function. Plasma troponin, a marker of myocardial damage, was significantly higher in controls than that of 2.5 and 10 mg/kg cyclosporine treatment groups.

Conclusion: We first demonstrated that the post-resuscitation administration of cyclosporine caused dose-related preservation of cardiac function in newborn piglets following asphyxia-reoxygenation.

PROBING STRUCTURAL TRANSITIONS IN SUPEROXIDE DISMUTASE 1, A PROTEIN WITH PRION-LIKE TEMPLATE-DIRECTED MISFOLDING ACTIVITY

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of motor neurons characterized by progressive weakness leading eventually to paralysis and impairment of respiration. A proportion of familial ALS cases have been linked to structure-altering mutations in the protein superoxide dismutase 1 (SOD1), a free radical defense enzyme that in disease may undergo a toxic gain-of-function resulting in increased production of reactive oxygen species. Recent evidence has demonstrated that template-directed misfolding of SOD1 occurs in a fashion analogous to prion conversion, which may contribute to the pathogenesis of ALS.

Methods: In this study, the molecular determinants of the SOD1 misfolding process were investigated by all-atom molecular dynamics simulation (MDS), and molecular modeling of thermodynamic and electrostatic stability.

Results and Conclusions: Regions of instability in SOD1 were identified by calculating the free energy of unfolding for all contiguous subsequences of the protein. The deformability of these unstable regions was explored by steered MDS to confirm their reduced resistance to forced unfolding relative to regions with greater predicted stabilities. A comprehensive inventory of electrostatic interactions between charged groups in wild-type and mutant SOD1 was also completed using continuum electrostatics calculations and a spatially-varying dielectric field extracted from equilibrium MDS of SOD1. This information is used to describe the structural changes that take place on SOD1 misfolding.

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DEFINING THE EP RECEPTOR SUBTYPES THAT MEDIATE THE ANTI-INFLAMMATORY EFFECTS OF PROSTAGLANDIN E₂ IN HUMAN MYOMETRIAL SMOOTH MUSCLE CELLS

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Background/Purpose: Despite decades of research, the mechanism underlying the onset of human labour remains poorly understood. Prostaglandin E₂ (PGE₂) production is increased in human myometrium with labour onset and PGE₂ analogues are used clinically to induce labour. PGE₂ elicits its effects via interaction with the EP receptors, all four of which are present in human myometrium. EP₁ and EP₃ typically mediate excitatory responses (e.g. smooth muscle contraction) whereas activation of EP₂ or EP₄ promotes inhibitory effects in most tissues.

Methods: Human myometrial smooth muscle (HMSM) cells were isolated from uterine samples obtained from term Caesarean section procedures. Cells were treated with EP pharmacological agents in the presence and absence of IL-1 β . Elaboration of IL-8, an inflammatory chemokine known to be up-regulated with the onset of labour, into the culture medium was measured by ELISA.

Results: Treatment of HMSM cells with IL-1 β caused a significant increase in IL-8 output compared to non-stimulated controls. Addition of PGE₂ prior to IL-1 β treatment resulted in a concentration-dependent repression of IL-8 output. At a maximally-effective concentration of PGE₂ (1 μ M), IL-8 output was repressed by over 60%. Treatment with EP₂ and EP₄ agonists also repressed IL-8 output. In contrast, use of an EP₃ agonist resulted in a further augmentation of chemokine output.

Conclusion: PGE₂ represses IL-1 β -induced inflammatory chemokine output from HMSM cells. Our data suggest PGE₂ elicits this anti-inflammatory effect in part by the EP₂ and EP₄ receptor subtypes. More targeted therapy may improve labour induction and reduce the rate of emergency Caesarean section deliveries.

PLASTICITY OF MOUSE ENTERIC SYNAPSES THROUGH RETROGRADE ENDOCANNABINOID AND PURINERGIC SIGNALLING

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Background & Aims: The enteric nervous system (ENS) is a complex neuronal network that possesses extensive synaptic connections which integrates information and provides appropriate outputs to coordinate the activity of gastrointestinal (GI) smooth muscle. For neurons within the ENS, the properties and regulation of the cell soma excitability is well understood. However the regulation of the ENS synapse is understood only superficially.

Methods: Intracellular microelectrode recordings were obtained from mouse myenteric plexus neurons. Interganglionic fibres were stimulated with a concentric stimulating electrode to elicit synaptic events on to the recorded neuron. Differences between spontaneous and evoked fast synaptic transmission was examined within preparations from cannabinoid receptor 1 (CB₁) deficient mice (CB₁^{-/-}) and wild-type littermate controls (WT).

Results: Presynaptic localization of the CB₁ receptor was demonstrated via colocalization with synaptotagmin. A greater proportion of CB₁^{-/-} neurons received spontaneous fast excitatory post-synaptic potentials (fEPSP) than neurons from WT preparations. Furthermore, CB₁ agonist WIN55,212-22 could depress WT synapses without any effect on CB₁^{-/-} synapses. Activity dependent liberation of a retrograde purine messenger was demonstrated to facilitate synaptic transmission in CB₁^{-/-} mice.

Conclusions: Here we show that endocannabinoids acting at CB₁ receptors inhibit transmitter release at enteric synapses and depress synaptic strength both basally and in an activity-dependent manner. These actions of endocannabinoids explain accelerated intestinal transit observed in the absence of CB₁ receptors. Furthermore, we reveal a novel activity-dependent purinergic retrograde synaptic modulation that increases synaptic strength. These findings suggest therapeutic avenues at the enteric synapse for the treatment of GI motor disorders.

ICILIN, AN AGONIST OF COLD ACTIVATED TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS, REDUCES INFLAMMATORY RESPONSE IN MICE COLON

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Background/Purpose: TRPA1 and TRPM8 are recently identified members of the transient receptor potential family of ion channels that plays an important role in detecting mild and painful cold temperatures. TRPA1 is activated by temperatures below 18°C and TRPM8 is reported to be activated at temperatures below 25°C. These channels are also activated by a chemical compound known as icilin. Since cold temperature is commonly used as local anti-inflammatory treatment, we hypothesised that icilin would diminish colitis in mouse models of colonic inflammation.

Methods: Colonic inflammation in C57BL6 mice was induced by treatment with either 2.5% dextran sodium sulphate (DSS) solution as drinking water or tri-nitro-benzene sulphonic acid (TNBS, 2mg in 100ul of 40% ethanol, intracolonic). Colitis was allowed to develop for 7 days and icilin (100µg in 3% DMSO/saline, intraperitoneally) was administered daily. Seven days after induction of colitis, macroscopic damage score, bowel thickness and myeloperoxidase (MPO) activity were measured

Results: DSS or TNBS challenge induced potent colonic inflammation in mice leading to significant increases in bowel thickness, macroscopic damage scores and MPO activity. However, DSS- or TNBS-induced increase in bowel thickness and macroscopic damage scores were significantly reduced in mice that received daily icilin treatment. Lastly, mice challenged with TNBS showed significantly reduced MPO activity when treated with icilin as compared to mice treated with vehicle.

Conclusion: We conclude that icilin confers protection against development of colitis in mice. Therefore, agents that can activate TRPA1/TRPM8 channels may represent novel therapeutics for inflammatory bowel diseases in the future.

AUGMENTED IMAGE-GUIDANCE FOR PERCUTANEOUS AORTIC VALVE REPLACEMENT

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Background and Objectives: In percutaneous aortic valve replacement, a valve mounted on a stent is inserted through a catheter trans-apically or trans-femorally. This procedure is primarily guided by fluoroscopy. Poor anatomical visualization in these images can lead to mal-alignment, damage to adjacent cardiac structures and coronary obstruction. In this study, we attempt to augment fluoroscopy images through the use of anatomical information derived from transesophageal (TEE) ultrasound to create an improved image-guidance system.

Methods: US-fluoroscopy registration is achieved by tracking the TEE probe in 3D using single-perspective pose estimation [1] [2]. The accuracy of a point-based algorithm and an intensity-based algorithm are tested for this application using simulations and experimental tracking experiments. The image-based tracking technique provides high accuracy in the setting of a conventional OR which may cause distortions with magnetic tool tracking. Localization of the US image plane with respect to the probe is determined using a modified Z-bar calibration [3]. Determined image transforms are applied to overlay US points onto the fluoroscopy image. We assess the accuracy of our registration by imaging a point phantom, and apply our algorithm to clinical images.

Results: Our system is able to register fluoroscopy and US images with an RMS accuracy of 1.24mm. This represents a clinically acceptable error.

Conclusions: It is feasible to provide augmented image-guidance using single-perspective TEE probe tracking. Intra-operative US images can be overlaid upon fluoroscopy images to provide anatomical context. This fast and robust registration technique has the potential to be implemented in the operating room in real-time.

[1]Hoffmann, K.R., Esthappan, J.: Determination of three-dimensional positions of known sparse objects from a single projection. *Med Phys* 24(4) (1997) 555–64 7.

[2]Penney, G.P., et al: A comparison of similarity measures for use in 2-D-3-D medical image registration. *IEEE Trans Med Imaging* 17(4) (1998) 586–95

[3] Gobbi, D.G., Comeau, R.M., Peters, T.M.: Ultrasound Probe Tracking for Real- Time Ultrasound/MRI Overlay and Visualization of Brain Shift. In: MICCAI'99.

Volume LNCS 1679/1999 of Lecture Notes in Computer Science. (1999) 920–927

SUB-CELLULAR LOCALIZATION OF Y-BOX PROTEIN 1 REGULATES PROLIFERATION, INVASION, AND INCREASED MESENCHYMAL PHENOTYPE IN ASTROCYTOMAS

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Background: Y-Box-Protein-1 (YB1) is a DNA/RNA-binding protein mandated for embryonic development and implicated in cancer progression. We previously established elevated YB1 levels in Pediatric Glioblastoma (pGBM), an aggressive high-grade brain tumor, possibly driving oncogenesis in this cancer. We investigated herein the effects of stable knock-down or ectopic expression of YB-1 in 4 GBM cell lines and in Htert-immortalized astrocytes.

Methods: YB1- expression was stably silenced or overexpressed in SF188, U251, U87 GBM cell lines and in H-tert immortalized astrocytes. Effects of overexpressing or silencing YB1 were assessed on cell proliferation and mesenchymal properties (migration, expression of mesenchymal markers).

Results: Silencing YB1 in GBM cell lines leads to increased proliferation (monolayer/soft agar), where the residual YB1 is localized in the nucleus. YB1 overexpression in GBM cell lines doesn't have any effect on cell proliferation, and the ectopic YB1 is mostly expressed in cytoplasm. In H-tert immortalized astrocytes, silencing YB1 decreased proliferation, and residual YB1 was mainly cytoplasmic. Silencing YB1 in NHA also decreased migration while ectopic expression increased migration, in addition to increased mesenchymal markers expression. Therefore, nuclear YB1 functions to promote cell growth, whereas cytoplasmic YB1 is more important in inducing mesenchymal phenotype and possibly growth inhibition.

Conclusion: Caution should be taken in targeting YB1 for therapeutic intervention. It may be feasible in early astrocytomas when disruptions of normal signaling pathway haven't occurred. In late stage astrocytomas when multiple molecular signaling pathways have been altered, targeting YB1 may cause tumor cells to be more proliferative.

FUNCTIONAL SIGNIFICANCE OF BLOOD OXYGEN LEVEL DEPENDENT (BOLD) IMAGING IN PATIENTS WITH CORONARY ARTERY DISEASE – A VALIDATION STUDY USING FRACTIONAL FLOW RESERVE

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Background/Purpose: Blood oxygen level-dependent (BOLD) cardiac MRI (CMR) uses the signal generated by haemoglobin to directly measure tissue oxygenation and may represent a non-invasive method to assess myocardial ischemia in patients with coronary artery disease (CAD). The aim of this study was to validate whether BOLD CMR can detect and quantify alterations in myocardial oxygenation in CAD.

Methods: BOLD CMR scans were performed in patients that had evidence of ischemia on a prior myocardial stress test. BOLD images were captured during rest and adenosine-induced coronary hyperaemia. The mean BOLD signal intensity (SI) percent changes were calculated between rest and hyperemia and were compared to intracoronary fractional flow reserve (FFR) assessed on angiography. FFR is the current gold standard to assess the extent of coronary occlusion (FFR <0.80 indicates significant stenosis).

Results: 18 patients (61±9y, 15 males) were available for analysis. Five were normal controls (FFR ≥ 0.80) and 13 had FFR values <0.80. There was a significant difference in the study population, whereby mean BOLD SI percent change was less in patients with abnormal FFR values (-8.27±2.84% SEM), in comparison to patients with normal FFR values (5.84±2.54% SEM); p=0.011.

Conclusion: This pilot study found that BOLD-sensitive CMR can detect changes in oxygenation in patients with CAD. Our preliminary data suggests that BOLD-sensitive CMR may allow for a non-invasive approach to directly assess myocardial ischemia in patients with coronary artery disease.

IMMUNOMODULATORY PEPTIDE IDR-1018 ATTENUATES LOCAL AND SYSTEMIC INFLAMMATION IN A MURINE DSS-COLITIS MODEL

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Background: Innate defence regulator (IDR) peptides are immunomodulatory agents that enhance bacterial clearance by increasing leukocyte recruitment to infection sites while simultaneously suppressing harmful inflammation. We hypothesized that IDRs might be efficacious in attenuating inflammation in a murine model of inflammatory bowel disease (IBD) where inappropriate inflammation is triggered by intestinal microflora.

Methods: Colonic inflammation was induced by addition of 2.75% DSS (w/v) to the drinking water of male C57BL/6 mice for 9 days. Mice were randomized to receive IDR-1018 (a lead anti-inflammatory peptide) on days 3 and 6. Weights were recorded daily, and serum was collected for measurement of Serum Amyloid A (SAA) by ELISA. RNA was isolated from colonic tissue at necropsy, and used to quantify chemokines by real-time PCR. Additional tissues were reserved for myeloperoxidase (MPO) assays to quantify neutrophil infiltration, as well as H&E staining for histological examination and disease scoring.

Results: Mice administered DSS showed progressive weight loss beginning on day 5. IDR-1018 slowed the overall weight loss trajectory, and significantly improved weights on days 8 and 9. Histological signs of colitis were markedly decreased in the distal colon, with a corresponding reduction in systemic inflammation (by SAA). IDR-1018 increased levels of colonic leukocyte chemoattractants, and enhanced neutrophil recruitment.

Conclusions: These data provide the first evidence that anti-infective IDR peptides might also be efficacious in inflammatory diseases. Further research is needed to determine if the protective effect of IDR-1018 in DSS-colitis is due to enhanced immune killing of intestinal microbiota, attenuation of inflammation, or both.

THE ROLE OF SHIP-2 IN CEACAM1-DEPENDENT EPITHELIAL CELL RESPONSES TO *NEISSERIA GONORRHOEA*

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Background: The Carcinoembryonic Antigen-related Cellular Adhesion Molecule-1 (CEACAM1) is expressed on the surface of epithelial cells, and acts as a receptor for *Neisseria gonorrhoeae*. The co-inhibitory function of CEACAM1 has been attributed to its cytoplasmic domain-localized immunoreceptor tyrosine-based inhibitory motif (ITIM) recruiting tyrosine phosphatases that dephosphorylate components involved in cellular responses to pathogens.¹ Harnessing this potent immunosuppressive effect undoubtedly helps *N. gonorrhoeae* to evade host innate responses to infection. Recently, a bioinformatic screen suggested that CEACAM1 may recruit lipid phosphatases as well as the heretofore described tyrosine phosphatases; however, this function has not yet been investigated.² SHIP-2 is an SH2 domain-containing 5-inositol phosphatase considered to be a negative regulator of activating phosphoinositide pathways such as the PI3K pathway.

Methods: We sought to determine if SHIP-2 associates with CEACAM1 in response to infection by *N. gonorrhoeae*. We assessed the interaction of SHIP-2 with CEACAM1 in an epithelial cell model using immunofluorescence-based imaging.

Results: SHIP-2 colocalizes with *N. gonorrhoeae*-bound CEACAM1 but not with bacteria adhering to other receptors. Consistent with a model that the ITIM of CEACAM1 is phosphorylated during cellular activation and then recruits SHIP-2 via its SH2 domain, the phosphatase inhibitor, pervanadate, caused SHIP-2 colocalization with the gonococci to persist whereas the addition of a Src kinase inhibitor, PP2, decreased SHIP-2 association.

Conclusion: These results support a CEACAM1-dependent regulation of phosphoinositide pathways via recruitment of SHIP-2 in response to infection by *N. gonorrhoeae*, thereby contributing to neisserial inhibition of epithelial cell immune responses.

¹ Slevogt, H. *et al.* CEACAM1 inhibits Toll-like receptor 2-triggered antibacterial responses of human pulmonary epithelial cells. *Nat.Immunol.* **9**, 1270-1278 (2008).

² Shlapatska, L. M. *et al.* CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. *J.Immunol.* **166**, 5480-5487 (2001).

DECREASED PLACENTAL FOLIC ACID TRANSPORT AFTER HEAVY ALCOHOL EXPOSURE

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Background: During pregnancy, the placenta concentrates folate into the fetal circulation so that fetal levels are 2-fold higher than maternal. Folate is vital to proper fetal development, methylation status, and detoxification of formic acid (the toxic metabolite of methanol that has been detected in cord blood of alcohol-exposed infants). Animal and *in vitro* studies have suggested that alcohol impairs placental transport of folate. We hypothesize that folate transfer to the fetus is decreased after heavy alcohol exposure and this may, in part, contribute to the deficits observed in the fetal alcohol spectrum disorders (FASDs).

Methods: Serum folate was measured in maternal and cord blood at delivery from alcohol-abusing mothers and controls.

Results: Women included in the alcohol-group reported daily alcohol consumption or greater than 8 drinks per week. The fetal:maternal serum folate ratio was <1.0 in over half of the alcohol-exposed pairs (median 0.84, range 0.24 - 4.84) (n=25), whereas all of the controls were >1.0 (median 1.80 range 1.23 - 2.99)(n=8). Mean folate in cord samples was lower in alcohol-exposed cord than in the controls (29.67 ± 14.43 vs 44.04 ± 10.86, p=0.01).

Conclusions: To our knowledge, this is the first study to show that folic acid transport to the human fetus is compromised in pregnancies affected by alcohol abuse. Future studies will determine if these fetal folate levels are associated with deficits under the FASDs in order to determine if proper folate supplementation can reduce these deficits.

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ABERRANT PRIMARY CILIA ARE FOUND IN HUMAN ASTROCYTOMA/GLIOBLASTOMA CELLS

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Background: Primary cilia are non-motile sensory cytoplasmic organelles implicated in various cell functions including sensation of fluid flow, growth factor signaling and cell cycle progression. Defects in formation/function of these structures underlie a variety of human diseases including obesity, ataxia and mental retardation. Primary cilia are linked to cell cycle regulation suggesting they may play a role in tumor metastases. The expression and function of primary cilia in cancer cells has become a focus of attention but has not been studied in astrocytomas/glioblastomas. We hypothesized that defects in ciliogenesis are common in cells derived from astrocytomas/glioblastomas and likely contributes to the phenotype of malignant cells.

Methods: Cultured normal astrocytes and five astrocytoma/glioblastoma cell lines were examined for primary cilia structure using indirect immunofluorescence and electron microscopy. Monospecific antibodies were used to detect primary cilia and map the relationship between the primary cilia region and sites of endocytosis.

Results: Expression of primary cilia in normal astrocytes is cell cycle related and each cilium extended into the extracellular environment *via* a cilium-pit, a site of endocytosis. In contrast, fully formed primary cilia were completely absent or abnormal in each of the astrocytoma/glioblastoma cell lines. Disruption of ciliogenesis in astrocytoma/glioblastoma cells prevented the formation of a site for endocytosis-based signaling.

Conclusions: The formation of the primary cilium was disrupted in cells derived from astrocytoma/glioblastoma tumors providing the first evidence that altered primary cilia may be part of the malignant phenotype. We demonstrated that early stages of primary cilia formation are critical for ciliogenesis.

DEVELOPING A VECTOR TRACKING STRATEGY TO ANALYZE THE CLONAL EXPANSION POTENTIAL OF NORMAL AND MALIGNANT HUMAN MAMMARY EPITHELIAL CELLS IN VIVO

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Background/Purpose: Breast tumors are known to be heterogeneous in their cellular composition and recent evidence suggests that only a limited subset of biologically distinct breast tumor cells actually possess tumor-forming potential, hence their designation as 'breast cancer stem cells'. On the other hand, the genomic instability of breast cancers revealed by a variety of analytical methods has suggested that these tumorigenic cells are also constantly diverging and changing in their frequency and properties.

Methods: To elucidate these proposed types of heterogeneity within breast tumors, we are developing a method for characterizing and comparing the *in vivo* clonal growth properties of xenografted human mammary cells isolated from normal and malignant tissue. In a first experiment, a lentiviral vector (Lenti-MNDUS-GFP) was used to identify the parameters that affect the efficiency of transducing normal primary human epithelial cells.

Results: The results showed that a 3 hour exposure of the cells to a high titre virus preparation in the presence of protamine sulfate, gave a 60% transduction efficiency of the cells present 3 days later when these were cultured on collagen-coated tissue culture plates (% GFP+ cells). However, this transduction efficiency was also accompanied by evidence of significant toxicity.

Conclusion: Further optimization of this transduction protocol to retain transduction efficiency and stem cell activity will allow for this tracking strategy to be used in conjunction with quantitative assays for monitoring breast cancer stem cell growth in xenografted immunodeficient mice.

INVESTIGATING CRMP4 FUNCTION IN CNS NERVE REGENERATION

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Background/Purpose: CNS neurons fail to regenerate following injury, in part due to the expression of inhibitory molecules including myelin associated inhibitors (MAIs) and chondroitin sulfate proteoglycans (CSPGs) in the glial scar. MAI and CSPG signalling converge to activate the cytosolic protein RhoA. RhoA antagonists promote neuronal survival and regeneration in animal models of nerve injury. However, the widespread role of RhoA in multiple cellular processes and cell types may limit its potential as a therapeutic target. In an attempt to discover more specific therapeutic targets to promote nerve regeneration, our lab previously identified CRMP4b (Collapsin Response Mediator Protein 4b) as a cytosolic phosphoprotein that interacts with RhoA to mediate neurite outgrowth inhibition. Blockade of the RhoA-CRMP4b interaction with a competitive peptide C4RIP (Crmp4b-RhoA Inhibitory Peptide) attenuates neurite outgrowth inhibition on myelin and CSPG inhibitory substrates.

Methods: To evaluate the therapeutic potential of C4RIP in vivo, we used an adeno-associated-virus (AAV) to express C4RIP in retinal ganglion cells (RGCs) and measured their ability to regenerate after optic nerve injury. We are currently developing a readily deliverable cell-permeable form of C4RIP (TAT-C4RIP) to further investigate the ability of C4RIP to promote nerve regeneration.

Results: AAV-mediated C4RIP expression in RGCs did not promote regeneration following optic nerve injury due to insufficient protein expression. We are currently investigating the ability of TAT-C4RIP to modulate regeneration following optic nerve injury.

Conclusion: These studies will continue to evaluate the role of CRMP4 in nerve regeneration and the potential of C4RIP as a therapeutic agent to facilitate recovery after CNS injury.

THE EFFICACY OF SCLEROTHERAPY WITH A SOLUTION OF DEXTROSE AND LIDOCAINE TO TREAT CHRONIC ACHILLES TENDINOPATHY: 1 YEAR FOLLOW-UP OF A RANDOMIZED CONTROLLED TRIAL

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Background/Purpose: Describe the outcomes of adults with chronic painful Achilles tendinopathy involved in a randomized controlled trial of sclerotherapy of neovessels with 25% dextrose and 1% Lidocaine.

Methods: After 12 weeks (up to 3 injections) in the RCT, each subject was unblinded to their treatment allocation, and subjects initially allocated to placebo (1% Lidocaine) were offered active injection. Subjects initially allocated to the active injection were allowed up to 2 additional injections if neovessels and pain persisted. Regardless of allocation, subjects (9 females and 7 males) were followed for a minimum of 12 months following their final injection. All subjects who received active injection were pooled for analysis.

Results: After final active injection, the mean difference in VISA-A score in participants who crossed over from the placebo group was 27.00 (7.16, 46.84). After the final sclerotherapy session, all participants were satisfied with treatment with a mean VISA-A score of 86.25 (80.19, 92.31). Ten remained satisfied with treatment at 6 months and seven participants at 12 months. At 6 months and 12 months, the mean difference in VISA-A from the final treatment session in satisfied participants was 3.70 (-2.74, 10.14) and -0.14 (-10.21, 9.92) respectively, whereas the mean difference in the unsatisfied participants was -12.50 (-21.45, -3.55) and -5.56 (-17.47, 6.36) respectively.

Conclusion: The long term effects of sclerotherapy appear to decrease over the course of 1 year. It is suggested that sclerotherapy be evaluated for its efficacy and safety as a maintenance therapy option whereby patients are permitted further injections when symptoms resurface.

LONGITUDINAL RELATIONSHIPS BETWEEN RELIGIOUS ATTENDANCE AND SPIRITUALITY WITH SUICIDAL IDEATION AND ATTEMPTS: FINDINGS FROM THE BALTIMORE EPIDEMIOLOGIC CATCHMENT AREA STUDY

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Background/Purpose: Few longitudinal studies exist examining the relationships between religion/spirituality and suicide attempts. Most studies are cross sectional and do not adjust for potential confounding variables. We conducted a study to examine the longitudinal relationships of religious worship attendance and seeking spiritual comfort with suicidal ideation and attempts in a community sample.

Methods: Data are drawn from waves 3 (conducted in 1993) and 4 ((conducted in 1996) (N=1,071)) of the Baltimore Epidemiologic Catchment Area study. Respondents were age 30 and older. Logistic regression was used to examine the relationship between wave 3 religious worship attendance and self-report seeking spiritual comfort with wave 4 incident suicidal ideation and attempts. Regression were then adjusted for the effects of socio-demographic factors, social supports and comorbid mental disorders.

Results: Respondents who attended religious services at least once per year had decreased risk of an incident suicide attempt independent of the effects of comorbid mental disorders and social supports (Adjusted odds ratio = 0.22, 95% Confidence Interval: 0.07-0.63). No significant relationships were found between religious worship attendance and suicidal ideation. Seeking spiritual comfort was not associated with suicidal ideation and attempts.

Conclusion: These results suggest that religious attendance is a protective factor against suicide attempts.

¹ Rasic DT, Belik SL, Elias B, Katz LY, Enns M, Sareen J, Swampy Cree Suicide Prevention Team. Spirituality, religion and suicidal behaviour in a nationally representative sample. *J.Affect.Disord.* 2009; 114: 32-40.

² Baetz M, Bowen R, Jones G, Koru-Sengul T. How spiritual values and worship attendance relate to psychiatric disorders in the Canadian population. *Can.J.Psychiatry* 2006; 51: 654-61.

MURINE PLACENTAL LACTOGEN-1A GENE EXPRESSION IS AFFECTED BY MATERNAL FEEDING IN LATE PREGNANCY

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Background/Purpose: The placenta is an important endocrine organ that produces hormones that can be found in both the maternal and fetal circulations. In mice, there are 22 placenta-specific, Prolactin-related hormones, including the placental lactogens (PLs). One of the major functions of PLs is to modulate glucose levels and prevent gestational diabetes during pregnancy. As such, we hypothesized that PL gene expression is sensitive to nutrient levels in the blood and would therefore change in response to maternal feeding.

Methods: To test this, we analyzed the mRNA levels of *Pl1* and *Pl2* in the murine placenta at four timepoints throughout the day in late pregnancy. In addition, dams were fasted overnight for 6 or 12 hours, during their normal feeding period, in order to assess whether *Pl* gene expression was altered by food deprivation.

Results: *Pl1*, but not *Pl2*, transcripts were found to increase in both the junctional zone and labyrinth of the placenta over the course of the night. *Pl1* gene expression increased in response to maternal feeding during the night, but expression levels increased earlier in the labyrinth as compared to the junctional zone. *Pl1* mRNA levels also failed to increase in the junctional zone when the mice were fasted.

Conclusion: In conclusion, *Pl1* gene expression in the mouse placenta is responsive to maternal changes in nutrition. These findings indicate that some placental hormone levels are affected by maternal feeding and may play a role in ensuring a steady supply of nutrients to the fetus.

POLYOMAVIRUS IN NON-MELANOMA SKIN CANCER

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Background: Non-melanoma skin cancer (NMSC) is the most common form of cancer worldwide and affects nearly 100,000 Canadians per year. While basal cell carcinomas (BCCs) are typically slow growing and rarely metastasize, squamous cell carcinomas (SCCs) are associated with higher mortality rates. In solid organ transplant recipients (OTRs), the risk for developing a SCC is 64-250 times that of the general population (Transplantation 49:506).

Oncogenic viruses have been implicated in certain types of NMSC, including SCC and Merkel cell carcinoma (Science 319:1096; J Invest Dermatol 129:2868). Polyomaviruses are small, non-enveloped DNA viruses which are widespread in nature. Initial infection is usually occult; however, viral reactivation of the BK polyomavirus is associated with graft rejection and, possibly, long-term malignancy in renal transplant recipients.

Herein, we study the association between polyomaviruses and NMSC in both OTRs and immunocompetent patients.

Methods: The presence of polyomavirus in NMSC paraffin-embedded skin sections was detected with a monoclonal antibody to SV40 large T antigen. Using indirect immunofluorescence techniques, fluorescence intensities were graded semi-quantitatively.

Results: SV40 expression was noted in BCCs (8/10), SCCs from immunocompetent patients (8/10) and SCCs from transplant recipients (4/5). In normal human skin, expression was typically absent to weak. The frequency and intensity of staining were clearly increased in tumour tissue as compared to normal skin.

Conclusion: Our data establish an unprecedented association between polyomavirus expression and NMSC. By shedding light on the molecular mechanisms underlying skin carcinogenesis, our findings may point to novel therapeutic targets for the prevention and treatment of NMSC.

ADAM10-MEDIATED PROCESSING OF GPNMB/OSTEOACTIVIN RELEASES AN EXTRACELLULAR DOMAIN WITH ANGIOGENIC PROPERTIES

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Background: Glycoprotein non-metastatic B (GPNMB) is a transmembrane protein that is expressed in 41-74% of breast cancers. It promotes migration, invasion and metastasis of breast cancer cells (1), is associated with shorter recurrence-free and overall survival times, and is most commonly expressed among patients with breast tumors of the basal/triple-negative subtypes who are not amenable to currently available targeted therapies (2). For these reasons GPNMB is an attractive target for therapeutic intervention in breast cancer; indeed, a GPNMB-targeted antibody-drug conjugate (CDX-011) has shown impressive clinical response among breast cancer patients and is currently being investigated in Phase IIb clinical trials. Despite its promise as a therapeutic target in breast cancer, little is known about the functional role of GPNMB the primary tumor microenvironment.

Methods: We have employed *in vivo* tumor growth and matrigel plug assays along with immunohistochemistry to analyse the effects of GPNMB on tumor growth. We used *in vitro* migration assays to assess function of the extracellular domain (ECD) of GPNMB and *in vitro* siRNA-mediated knockdown of ADAM molecules to identify a GPNMB sheddase responsible for ECD liberation.

Results: In this study we show that GPNMB promotes the outgrowth of mammary tumors *in vivo* and enhances their microvascular density. The GPNMB ECD is shed by breast cancer cells and is capable of inducing endothelial migration. Finally, we have implicated ADAM10 as a novel sheddase for GPNMB.

Conclusion: We have described ADAM10-mediated shedding of GPNMB ECD as a novel mechanism by which GPNMB can promote angiogenesis in breast cancer.

(1) A.A.N. Rose, A.A. Grosset, Z.F. Dong, *et. al.* (2010) **GPNMB is an independent prognostic indicator of recurrence and a novel therapeutic target in breast cancer.** *Clin. Cancer Res.* 16(7):2147-56.

(2) A.A.N. Rose, F. Pepin, C. Russo, *et. al.* (2007) **Osteoactivin promotes breast cancer metastasis to bone.** *Mol. Cancer Res.* 5(10): 1001-1014.

MEASURING OPPRESSION AND OTHER DETERMINANTS OF DEPRESSION AMONG PREGNANT ABORIGINAL WOMEN IN THE CALGARY AREA : STUDY DESIGN

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Background: Depression is more prevalent among women relative to men, and among Aboriginal populations relative to other Canadians. Depression during pregnancy carries serious maternal and child health consequences. Research on the determinants of depression among Aboriginal women is limited. Socioeconomic status and life stress are associated with depression in the epidemiologic literature; while these factors likely contribute, they may not fully account for depression in this population. Aboriginal women experience intersecting forms of oppression from race, gender and the intergenerational trauma of colonization. Empirical research examining the role of oppression in Aboriginal women's health is lacking, despite theoretical recognition.

Purpose: The proposed study uses a mixed-methods approach to assess the determinants of depression in this population, with a focus on comprehensively measuring oppression.

Proposed design: Existing frameworks and scales relevant to oppression will be examined in a systematic review. Qualitative interviews will be conducted with pregnant Aboriginal women in Calgary (Alberta) and the surrounding First Nations reserves. Based on the existing literature and the insight gained from the qualitative interviews, a conceptual framework will be proposed. A survey questionnaire to comprehensively assess this hypothesized framework will be designed, pilot-tested and conducted with another sample of pregnant Aboriginal women. The survey data will be analyzed using regression methods, to quantitatively model associations between proposed determinants and depressive symptoms scale score.

Significance: A better understanding of depression's determinants can help in the design of effective interventions. Furthermore, a comprehensive measure for oppression would have multiple applications in Aboriginal population health research.

THE EFFECTS OF FATTY-ACID OVERSUPPLY ON SKELETAL MUSCLE MITOCHONDRIAL FUNCTION IN C57BL/6J MICE

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Background: The widespread prevalence of obesity is receiving considerable interest as it is linked to cardiovascular disease and Type II diabetes. Mitochondria play a central role in energy balance, thus they present a prime target in the study of obesity. We show that a high fat diet results in changes in mitochondrial function and hypothesize that mitochondrial function is able to recover from such lipid-induced stress.

Methods: Male C57BL/6J mice were fed a standard chow (Ch) diet, or a high-fat (HF) diet for 21 weeks (n=7/group). Both *soleus* and *gastrocnemius* muscles were used for high-resolution respirometry studies. Our current work uses an exercise intervention where the animals are exercised for 1 hour/day at 6.0m/min, 6 days/week over a period of 4-5 weeks in a monitored exercise wheel system.

Results: Significant differences were seen in the *soleus* and *gastrocnemius* in State 3 respiration (1.93 ± 0.16 vs 1.02 ± 0.12 ; 2.0 ± 0.21 vs 0.66 ± 0.05 in for Ch vs. HF in the *soleus* and *gastrocnemius* respectively). A significant decrease was also noted in State 4 respiration (1.04 ± 0.08 vs 0.70 ± 0.09 ; 0.86 ± 0.09 vs 0.26 ± 0.01 ; $p \leq 0.007$, in for Ch vs. HF in the *soleus* and *gastrocnemius* respectively). Preliminary studies on isolated mitochondria also confirm these results with decreased respiration per unit of mitochondrial mass.

Conclusion: Chronic lipid oversupply ultimately produces mitochondrial damage. Our ongoing studies are evaluating the effectiveness of exercise for potentially reversing this damage.

MAP KINASE ACTIVATION INCREASES BK POLYOMAVIRUS REPLICATION AND FACILITATES VIRAL PROPAGATION IN VITRO

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Background: BK virus is widely accepted to be the causative agent of polyomavirus associated nephropathy (PVAN). Reactivation of BK virus following renal allograft transplantation results in a lytic infection in tubular epithelial cells leading to tubulointerstitial inflammation, fibrosis, and graft loss. Despite the recent surge in recognition of BK virus-associated diseases, relatively little is known regarding the biology of the virus and the factors that regulate viral replication.

Methods/Results: BK virus replication was augmented in HEL-299 cells cultured in conditions that activated the MAP kinase, ERK1/2. To determine if MAP kinase activation increased BK virus replication, cells were treated with serum and phorbol-12-myristate-13-acetate (PMA). Serum and PMA stimulated large T-antigen expression and increased BK virus DNA replication. The effects of serum/PMA were directly related to MAP kinase signal activation as the MEK1/2 inhibitor U0126 reduced viral replication. PMA also increased cyclin D1 expression and inhibition of cyclin D1/CDK4 complex formation reduced BK virus infection. The PMA effect occurred independently of transcriptional activation of the viral NCCR. In HEL-299 cells, virus infection in high serum and PMA accelerated viral replication resulting in the production of high titer infectious BK virus in a fraction of the time required by conventional protocols.

Conclusions: MAP kinase signal activation increases BK virus replication. These findings are of great clinical significance as they shed light on the mechanisms of BK virus reactivation and/or replication in the pathogenesis of PVAN.

SURGICAL STRESS PROMOTES THE DEVELOPMENT OF CANCER METASTASIS BY A COAGULATION-DEPENDENT MECHANISM

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Background: Surgery precipitates a hypercoagulable state and has been shown to increase development of metastases in animal models. Coagulation facilitates the formation of microthrombi around tumor cell emboli (TCE) in microvasculature thereby inhibiting Natural-Killer(NK) cell mediated destruction. We hypothesize that pro-metastatic effect of surgery may be secondary to the postoperative hypercoagulable state.

Purpose: The aim was to determine if surgical stress promotes development of metastases by increased formation of TCE associated microthrombi resulting in decreased NK cell mediated destruction and to evaluate the ability of low-molecular-weight-heparin (LMWH) to inhibit pro-metastatic effect of surgery.

Methods: Surgical stress was induced in BalbC mice by partial hepatectomy, preceded by tail vein injection of colon cancer cells with or without perioperative anticoagulation with subcutaneous tinzaparin. Mice were euthanized at various timepoints and TCE were quantified. Fibrinogen and platelets were fluorescently labelled prior to surgical stress to evaluate TCE associated fibrin and platelet clots.

Results: Surgery resulted in significant increase in metastases while anticoagulation with LMWH abrogated this effect. Significant difference in metastatic foci was seen at 12h and 3d post surgery but not at earlier time points. Fibrin and platelet clots were associated with TCE significantly more frequently in mice that underwent surgery, as compared to mice with no surgery or pretreatment with LMWH. Platelet depletion led to incomplete attenuation of metastatic deposits in surgically stressed mice.

Conclusion: Surgery promotes the formation of fibrin and platelet clots around TCE and this appears to be the mechanism for the increase in metastases seen following surgery.

THE EFFECT OF SUPPLEMENTAL VITAMINS AND MINERALS ON THE DEVELOPMENT OF PROSTATE CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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Background/Purpose: To date many studies have investigated the roles of vitamins and minerals with respect to prostate cancer risk and advanced stages of the disease. The results of these studies vary, making the correlation between vitamins and minerals and prostate cancer difficult to interpret. In this study we systematically reviewed the literature and performed meta-analysis in an attempt to better understand and interpret the literature.

Methods: Pubmed, Embase, and the Cochrane database were searched using selection criteria to collect fourteen articles. Following the literature search, the articles were review by each of the two authors using the US Preventive Services Task Force Quality Rating Criteria. The articles were then organised based on Study Type, Exposure and Outcome and the Cochrane Collaboration software, Revman 5, was used for the meta-analysis.

Results: Neither the use of multivitamin supplementation nor the use of individual vitamin/mineral supplementation affected the occurrence of prostate cancer, or the occurrence of advanced/metastatic prostate cancer or death from prostate cancer, when the results of the studies were combined in a meta-analysis. Sensitivity analyses were also performed by running meta-analysis using just the higher quality studies and just the randomized controlled trials with still no associations found.

Conclusion: Overall, when all of the identified eligible studies were combined in meta-analyses there was no effect of any of the vitamins or multivitamins on the occurrence or severity of prostate cancer.

EXPLORING RISKY BEHAVIOUR: FACTORS RELATING TO COMMUNITY PERCEIVED CONNECTIONS BETWEEN ANIMAL AND HUMAN HEALTH AMONGST THE MAASAI OF EAST AFRICA

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Background/ purpose: Pastoralists of East Africa depend on their livestock for sources of wealth and food. However, this form of livelihood increases the risk for zoonotic disease and economic losses due to poor livestock production. This project sought to explore how the Maasai perceive the link between their own health and the health of their animals using brucellosis as a zoonotic exemplar.

Methods: All participants were recruited using either chain-referral or purposive sampling techniques. Participant observation was combined with 3 types of interviews: focus group interviews (n=3 with 70 participants), key informant interviews with local human and animal healthcare personnel (n=9), and individual interviews with patients confirmed to have brucellosis (n=4).

Results: Although participants did identify brucellosis as a threat to their health, participants did not identify brucellosis as a priority disease nor did they identify brucellosis as disease affecting animals. Conversely, local healthcare staff and government representatives listed brucellosis as a priority disease for both animals and humans. Varying descriptions of causality and initial use of traditional medicine were also found.

Conclusions: These findings could have serious implications for future intervention design and implementation within this population. The Maasai of Ngorongoro do not perceive their animals as a potential reservoir for brucellosis. This perception could be due to the differing clinical presentation and symptom profile in humans versus animals. Cross-species differences in clinical presentation may help explain why previous education based interventions have failed within Maasailand for zoonotic diseases such as brucellosis. Integration between human and veterinary public health and clinical services should be pursued.

CHARACTERIZATION OF TELOMERASE MUTATIONS ASSOCIATED WITH IDIOPATHIC PULMONARY FIBROSIS

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Background/Purpose: Telomeres are protective nucleoprotein structures found at the ends of linear chromosomes and are crucial for maintaining genomic stability in cells. Telomeric DNA shortens after each round of cell division but can be synthesized by telomerase, a reverse transcriptase that minimally consists of a reverse transcriptase catalytic subunit (TERT) and an RNA molecule (TR). Short telomeres and abnormal telomerase activity have been associated with several human diseases. Recently, human TERT mutations have been identified in patients with idiopathic pulmonary fibrosis (IPF), a fatal lung disease characterized by alveolar damage and fibrosis. These patients also exhibit shorter telomeres compared to age-matched controls; however, the underlying mechanism(s) behind this phenotype is still unclear. The objective of this study was to determine whether IPF-associated hTERT mutations would disrupt telomerase function.

Methods: Focusing on three IPF-associated hTERT mutations (hTERT V144M, R865C and R865H), a series of *in vitro* and cellular telomerase activity assays, nucleic acid binding assays and telomere length assays were used to characterize the effects of these mutants on telomerase function.

Results: Each hTERT mutation was found to affect telomerase activity and hTERT-DNA interactions in a unique manner. Expansion of these studies in human cells enabled us to show that V144 and R865 are important amino acids in hTERT, and mutating these residues result in a defect in telomerase function.

Conclusion: We have identified two hTERT residues critical for telomerase function. In addition, this study has provided some insight into how these hTERT mutations may contribute to the development of IPF.

THE ROLE OF TRANSFORMING GROWTH FACTOR ALPHA IN A MOUSE MODEL OF OSTEOARTHRITIS

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Purpose: No cures currently exist for osteoarthritis (OA). In an attempt to identify targets for disease modifying osteoarthritis drugs (DMOADs), our lab established a surgical model of OA to study gene expression changes in degenerating cartilage. Transforming growth factor alpha (TGF α) gene expression was upregulated in our model and further *in vitro* studies showed that TGF α suppressed chondrocyte expression of anabolic factors and increased expression of catabolic factors. We thus identified TGF α as a novel therapeutic OA target. The purpose of this project is to examine the role of TGF α in the development of OA *in vivo*.

Methods: Adult male *Tgfa* null mice and control littermates received either meniscotibial transection (MTX) or sham surgery. At 7 weeks post-surgery knee joint histopathology was scored and tissues were immunostained for disease markers. In addition, joint pathology was assessed in six month old mice in order to examine spontaneous OA.

Results: MTX surgery produced mild OA in mice after 7 weeks. *Tgfa* null mice had lower OARSI scores and expressed less MMP13 and type II collagen neopeptides than their control littermates. Male *Tgfa* null mice appeared to be protected against spontaneous OA when compared to controls, while there appeared to be no differences in disease progression between the female groups.

Conclusions: TGF α signaling plays an important role in osteoarthritis progression *in vivo* and should be investigated further as a potential target for DMOAD development.

IMPAIRED RENAL FUNCTION MODIFIES THE RISK OF SEVERE HYPOGLYCEMIA AMONG USERS OF INSULIN BUT NOT GLYBURIDE: A POPULATION-BASED NESTED CASE-CONTROL STUDY

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Background/Purpose: Little evidence justifies the avoidance of glyburide in patients with impaired renal function. We aimed to determine if renal function modifies the risk of hypoglycemia among patients using glyburide.

Methods: We conducted a nested case-control study using administrative records and laboratory data from Ontario, Canada. We included outpatients 66 years of age and older with diabetes mellitus and prescriptions for glyburide, insulin or metformin. We ascertained hypoglycemic events using administrative records and we estimated glomerular filtration rates (eGFR) using serum creatinine concentrations.

Results: From a cohort of 19,620 patients, we identified 204 cases whose eGFR was ≥ 60 ml/min/1.73m² (normal renal function) and 354 cases whose eGFR was < 60 ml/min/1.73m² (impaired renal function). Compared to metformin, glyburide associated with a greater risk of hypoglycemia in patients with both normal (adjusted OR 9.0, 95% CI 4.9 to 16.4) and impaired renal function (adjusted OR 6.0, 95% CI 3.8 to 9.5). We observed a similar relationship when comparing insulin to metformin; the risk was greater in patients with normal renal function (adjusted OR 18.7, 95% CI 10.5 to 33.5) compared to those with impaired renal function (adjusted OR 7.9, 95% CI 5.0 to 12.4). Tests of interaction showed that among glyburide users renal function did not significantly modify the risk of hypoglycemia, but among insulin users, impaired renal function associated with a lower risk.

Conclusions: In this population-based study, impaired renal function did not augment the risk of hypoglycemia associated with glyburide use.

VALPROIC ACID TRIAL IN CLL: EXPERIENCE AT CANCERCARE MANITOBA AND ITS MECHANISM OF EFFECT

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Background/Purpose: In chronic lymphocytic leukemia (CLL), Valproic acid (VPA) had been shown to induce apoptosis in CLL cells *in vitro* and enhance fludarabine-induced apoptosis. This action has been associated with the upregulation of the TNF-related apoptotic ligand (TRAIL) apoptotic pathway.

Methods: A phase II clinical trial was initiated at CancerCare Manitoba in order to examine VPA alone or VPA in combination with fludarabine in treating relapsed CLL. Peripheral blood samples were obtained at set time points and analyzed using immunocytochemistry and immunoblotting.

Results: 28 days of therapy with VPA alone resulted in no improvement in five CLL patients. In four patients that participated beyond cycle two, fludarabine was combined with VPA. Among them, three patients participated past cycle five, in all of whom there were decreases in WBC and node sizes. Levels of both histone 3-acetyl and histone 4-acetyl were observed to initially increase then fluctuate during the course of treatment with VPA alone. DR4 levels increased during the course of the treatment, while no significant changes in DR5 levels were observed. Quantitative PCR for a set of apoptotic genes revealed global upregulation of a large number of genes involved in apoptosis.

Conclusion: Our data show that VPA induces hyper-acetylation of histones and upregulates DR4 levels *in vivo*, thereby further activating the TRAIL apoptotic pathway. While VPA appears ineffective in treating CLL as a single agent, VPA may be used as an adjuvant to nucleoside analogue-based treatment regimen in order to sensitize the CLL cells for nucleoside analogue-induced apoptosis.