3rd Annual LDI Scientific Retreat

Friday May 18th, 2012

Scientific Abstracts

Student Oral Presentations

(listed by axis in alphabetical order)
Inflammasome-mediated activation of Caspase-1 and Caspase-6 in primary human neurons

Kaushal, V. and Leblanc, A.C.

Caspase-6, an effector cysteine protease, is up-regulated in Alzheimer disease (AD) neurons and causes axonal degeneration in the absence of cell death. The activation of Caspase-6 is regulated upstream by the inflammatory Caspase-1, a component of a multiprotein complex known as inflammasomes; consisting principally of a nucleotide-binding oligomerization domain (NOD)-like receptor, apoptosis-associated speck-like protein containing caspase recruitment domain (ASC) and Caspase-1. While inflammasome function and intrinsic innate immunity have been well investigated in macrophages, little is known in neurons. In this study we examine the role of intrinsic neuronal immunity in regulating Caspase-6 activation. Methods: mRNA expression of the main protein components of the inflammasomes were assessed by quantitative RT-PCR of primary human neurons. Purinergic P2X7 receptors (P2X7R) were targeted with the agonist benzylated ATP (BzATP) to induce the inflammasome. P2X7R-mediated pore formation was assessed by the uptake of the YO-PRO-1 dye. Caspase-1 and Caspase-6 activity was measured using the fluorogenic substrates Z-YVAD-AFC and Z-VEID-AFC, respectively. A cell free inflammasome system was developed to examine inflammasome function by measuring Caspase-1 YVADase activity in neuronal cytosolic fractions. Results: P2X7R was expressed in neurons but not in astrocytes. Treatment of neurons with BzATP induced a considerable uptake of the YO-PRO-1 dye and this uptake was completely inhibited by Brilliant Blue G, a specific P2X7R antagonist. Activation of the inflammasome by BzATP was confirmed by increased Caspase-1 activity. Quantitative RT-PCR showed that inflammasome components, IPAF-1, AIM-2, NALP1, and ASC mRNA were expressed in neurons but only the NALP1 mRNA increased with BzATP treatment. No NALP3 inflammasome receptor expression was observed. BzATP-induced a sequential increase of Caspase-1 YVADase activity and Caspase-6 VEIDase activity within 30 min and 1 hr of treatment, respectively. Blocking Caspase-1 with inhibitor Z-YVAD-fmk reduced Caspase-6 activity in BzATP-treated neurons, confirming that inflammasome formation and Caspase-1 activity are upstream of Caspase-6 activation. Using a cell free system to study inflammasome function, Caspase-1 YVADase activity was detected in the cytosolic fraction of human neurons. Inhibition with an antibody against NALP1 reduced Caspase-1 YVADase activity, validating that NALP1 inflammasome regulates Caspase-1 activity in human neurons. Conclusion: These results provide the first characterization of inflammasome components in neurons. Their presence implies that neurons have intrinsic innate immune mechanisms involved in Caspase-1 and Caspase-6 activation. These mechanisms could explain the Alzheimer brain associated increased levels of interleukin-1-beta, a cytokine produced only by Caspase-1.
JMJD2A promotes cellular transformation by blocking cellular senescence through transcriptional repression of the tumor suppressor CHD5

Mallette, F.A. and Richard, S.

Senescence is a cellular response preventing tumorigenesis. The Ras oncogene is frequently activated or mutated in human cancers. However, Ras activation is insufficient to transform primary cells. In a search for cooperating oncogenes, we identify the lysine demethylase JMJD2A (KDM4A). We show that JMJD2A functions as a negative regulator of Ras-induced senescence and collaborates with oncogenic Ras to promote cellular transformation. Inactivation of the p53 tumor suppressor is a critical event in Ras-mediated tumor formation. We show that JMJD2A expression contributed to the bypass of Ras-induced senescence by negatively regulating the p53 pathway. To identify the molecular mechanism behind the control of p53 activation by JMJD2A, we performed ChIP-on-chip and identified CHD5, a known tumor suppressor regulating p53 activity, as a target of JMJD2A. The expression of JMJD2A inhibits Ras-mediated CHD5 induction leading to a reduced activity of the p53 pathway. In addition, we show that JMJD2A cooperated with Ras and E1A in the transformation of primary fibroblasts and that JMJD2A is over-expressed in mouse and human lung cancers. Furthermore, depletion of JMJD2A in human lung cancer cell line A549 bearing an activated K-Ras allele is sufficient to trigger senescence and significantly reduces cellular proliferation. We propose that JMJD2A is a cooperating oncogene that represents a novel target for the development of therapeutics for Ras-expressing tumors. This work is supported by funds from the CIHR.
Shear Stress-induced Atherosclerotic Plaque Regression is Reversed By Matrix Metalloproteinase Inhibition

Simeone, S., Ebrahimian, T., Michaud, V. and Lehoux, S.

Atherosclerotic plaques usually form in regions of low blood flow, whereas vessels exposed to high shear stress tend to remain lesion-free. We hypothesized that exposing established atherosclerotic plaques to elevated shear stress leads to lesion regression by facilitating inflammatory cell movement within the plaque. We developed a surgical procedure in mice, the arteriovenous fistula (AVF), where the right carotid artery is anastomosed into the right jugular vein. LDLR-/- mice were placed on a high-fat diet, then divided into control, sham and AVF groups. Sham and AVF groups were kept on a high-fat diet for a further 4 weeks post-surgery. This procedure increases the shear stress in the artery, leads to plaque regression and increases plaque matrix metalloproteinase (MMP) activity. To understand the mechanism of this regression, we assessed the role of MMPs. Plaque MMP-9 expression was increased in AVF vs. control and sham (10.8%±1.4, 3.9%±0.7, 4.6%±1.1, respectively; p<0.0001). MMP-3 expression was increased in AVF vs. control (9.5%±1.5, 5.0%±0.8, respectively; p<0.05). MMP-2 expression was decreased in AVF vs. sham (5.7%±0.5, 11.3%±1.6, respectively; p<0.05). In addition, we overexpressed the tissue inhibitor of metalloproteinase (TIMP-1) in mice before surgery. Sham plaque size was not affected, however, the increased TIMP-1 prevented the reduction in plaque size in the AVF group (0.096±0.013 mm² vs. 0.047±0.008 mm², p<0.05). All groups had comparable lipid levels. There was no difference in smooth muscle cell and collagen content between groups. To further understand the role of MMPs, we devised an endothelial cell (EC)-macrophage co-culture system where the ECs were exposed to high (15 dynes/cm²) or low (0.5 dynes/cm²) shear stress. Macrophage migration exposed to EC effluent was performed using a scratch assay. There was a 2.6 fold increase in migration in the high flow condition compared to the low (p<0.05). When the cells were exposed to the MMP inhibitor drug, GM6001, migration was decreased 2 fold (p<0.05). Our findings suggest that MMPs play a role in shear stress induced- plaque regression. Shear stress acting on ECs may influence the cells within the plaque by increasing MMP expression allowing for better macrophage mobility and egress which are characteristics of regressing plaques.
Screening and Vaccination Strategies for Preventing Hepatitis B Related Morbidity and Mortality in Immigrants: A Cost-Effectiveness Analysis

Rossi, C., Oxlade, O., Schwartzman, K. and Greenaway, C.

During the last four decades, immigration to Canada has increased from areas where hepatitis B virus (HBV) is endemic. Most chronic HBV infections in immigrants are asymptomatic and many are unaware of their infection. As a result, immigrants are at greater risk of developing liver disease, compared to the Canadian-born population. Objectives: We conducted an economic evaluation of four incremental HBV screening and immunization strategies designed to identify susceptible or chronically infected adult immigrants, in order to provide vaccination or antiviral therapy to reduce the HBV burden in immigrant communities. Methods: A cost-effectiveness analysis was performed using a decision-analysis model involving a Markov process to model chronic HBV sequelae. Seroprevalence estimates were obtained from a systematic review. Transition probabilities, intervention and medical costs, and utilities were obtained from the published literature. Interventions were compared by calculating incremental cost-effectiveness ratios, defined as the additional health benefit of a strategy, measured in quality-adjusted life years (QALY) gained, with the next least costly, undominated strategy. Costs and outcomes were modeled over a 45-year period and discounted at a rate of 3% per annum. Results: The “Screen and Treat” strategy was found to be the most cost-effective strategy with a cost of $37,675/QALY gained, relative to the status quo of no screening or vaccination. The “Screen, Treat and Vaccinate” strategy was found to provide little added benefit, as it was expected to cost $698,844/QALY gained, relative to the Screen and Treat strategy. The “Universal Vaccination” and “Screen and Vaccinate” strategies were dominated by the Screen and Treat strategy, as they generated fewer QALYs than the former strategy. Sensitivity analyses demonstrate that the results are sensitive to the cost and efficacy of treatment. Conclusion: Screening immigrants for chronic HBV was found to be cost-effective and can contribute to reducing HBV-associated morbidity and mortality.
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Student Poster Presentations

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AGING
Prion protein (PrP) inhibits Bax-mediated cell death in human primary neurons and breast carcinoma MCF-7 cells. While the role of PrP in neuroprotection has been widely investigated, few studies have evaluated the role of PrP in cancer. PrP is associated with resistance to anthracycline-based chemotherapy in breast cancer and plays a role in resistance to tumor necrosis factor-alpha and TRAIL-induced apoptosis in MCF-7 (Meslin et al., Cancer Res. 2007). The objective of this study was to determine how ER stress increases PrP levels in breast cancer cells and assess the clinical relevance of this regulation. We first observed that three pharmacological ER stressors, Thapsigargin, Tunicamycin and Brefeldin A, increased PrP mRNA and protein levels. This increase was blocked by actinomycin D indicating a transcriptional response. Four ER stress response elements (ERSE) were identified in the human PRNP promoter and luciferase reporter assays confirmed their involvement in both basal and ER stress-induced PrP expression. Overexpression of ATF6α, sXBP-1, but not ATF4, increased PrP levels while silencing of ATF6α decreased the ER stress-induced PrP levels in cells. Chromatin immunoprecipitation (ChIP) assays confirmed the binding of ATF6α and sXBP-1 to the PRNP promoter region during ER stress. From a functional standpoint, siRNA-mediated silencing of PrP highlighted a protective role of PrP by delaying ER stress-induced apoptosis in MCF-7 cells. To evaluate the clinical relevance of ER stress-dependent regulation of PrP expression, we studied PrP levels by immunohistochemistry in human breast cancer tissue microarrays and correlated them with ER stress marker, BiP, levels. Average PrP intensity score was significantly higher in high BiP-expressing cores. PrP levels were also associated with higher tumor grade. Analysis of PrP mRNA levels from published studies further strengthened these findings by showing that PrP is associated with estrogen receptor-negative tumors, poorer prognostic and that its expression is significantly higher in the more aggressive basal cell lines than in luminal cell lines. Overall, this work characterizes ER stress-induced PrP expression in breast cancer MCF-7 cells and reports a protective role for PrP against ER stress-induced cell death. Correlating PrP with the ER stress marker BiP in human breast tumour tissue as well as analysis of published mRNA levels suggests this regulation could be clinically relevant.
Episodic memory is commonly assessed using word-learning lists like the California Verbal Learning Test–Second Edition (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000). The CVLT differs from many conventional memory tests in that it provides information beyond the total number of correctly recalled words; learning, semantic, and executive constructs are measured by variables such as semantic clustering, learning over trials, and recall consistency. Previous studies have examined the factor structure of the CVLT-II in controls (Donders, 2008) and individuals with traumatic brain injury (TBI; DeJong & Donders, 2009) using Confirmatory Factor Analysis (CFA), and AD patients (Delis et al., 2003) using Exploratory Factor Analysis (EFA). Studies using CFA indicate that the best fitting model for controls and TBI patients consisted of four factors including Attention Span, Learning Efficiency, Delayed Memory, and Inaccurate Memory. On the other hand, EFA conducted with AD patients indicates that immediate and delayed recall loaded onto a single factor for the controls, whereas these measures formed separate factors for the AD sample (Delis et al., 2003). This finding indicates that factor solutions derived using controls may conceal important cognitive distinctions that characterize patient populations. We examined the factor structure of the CVLT-II in a sample of 161 MCI patients (age: M=79.64 , sd=8.4, education: M=13.45, sd=3.2). Patients were recruited from the Jewish General Hospital Memory Clinic, and data was taken from the first exposure to the CVLT-II as part of a complete neuropsychological assessment. Four CFAs were conducted to compare the four previously hypothesized models for fit and parsimony. Results revealed that although the four-factor model (Attention Span, Learning Efficiency, Delayed Memory, and Inaccurate Memory) had the best fit, it did not meet criteria for fit and parsimony. Additionally, the chi-square statistic was significant, indicating that the model and the data were significantly different. In order to replicate the factor analysis conducted with AD patients an EFA was conducted on 17 variables (not all of the 20 variables used in the AD study were available in the database). Results of the EFA suggest a five-factor model for the MCI patients. Importantly, immediate and delayed recall formed two separate factors, similar to that seen with AD patients. Taken together, the findings indicate that the model which best fits the standardization sample of healthy adults is not applicable to an MCI sample, whose results are more comparable to previously reported factor structure seen in AD.
Bladder augmentation using scaffold model seeded with bone marrow-derived stem cells in animal model

El Yazami Adli, O., Wally, M., Loutochin, O., Galipeau, J. and Corcos, J.

Bladder tissue engineering is a promising avenue for tissue replacement in urology. We aim to evaluate bladder augmentation using bladder acellular matrices (BAMs) seeded with bone marrow-derived stem cells (MSCs) in animal model. Materials and methods: 72 Sprague-Dawley rats were divided as follows: 6 for harvesting MSCs, 22 for harvesting bladders, and 44 rats were subdivided into sham control, partial cystectomy (PC), augmentation with BAMs only and augmentation with MSCs seeded BAMs. At 1 and 6 months, we performed urodynamic studies before sacrificing the animals for histological analysis and immunohistochemistry. Results: Similar mean bladder capacities (MBC) were recorded in the normal control and sham control groups (0.79 ± 0.12 vs. 0.74 ± 0.15ml). The PC group had only 45% of their native bladder capacity at 1 and 6 months. The non-seeded group increased their MBCs by 55% compared to preoperative values at 1 month, and 65% at 6 months. MSCs-seeded BAMs bladders reached MBCs of 75% and 99% in comparison to the original precystectomy volume at 1 and 6 months respectively. Bladders grafted with MSCs-seeded BAMs showed a satisfactory muscularis propria development when compared to bladder augmented with BAMs only. Conclusions: Seeding MSCs onto BAMs is successful in animal model and very promising for bladder augmentation.
The Montreal Cognitive Assessment (MoCA) is a screening test developed to detect mild cognitive impairment (MCI; Nasreddine et al., 2005). The test assesses multiple cognitive domains with one or more short items. However, even within the same general cognitive domain, each item is designed to test a different cognitive function. Previous studies examining the factor structure of the MoCA have used either a combined sample of Normal Elderly Controls (NEC) and cognitively impaired individuals, or MCI patients and individuals with different forms of dementia. These studies report a one-factor, two-factor and, most recently, a six-factor structure of the MoCA (Duro et al., 2010; Freitas et al., 2012; Koski et al., 2009). However, combining individuals with varying levels of cognitive functioning may disguise important performance differences between the groups. This study examined whether the factor structure underlying performance on the MoCA varies across NECs, MCI and AD patients. To do so, we performed an exploratory factor analysis using a large sample of participants (223 NECs, 320 MCIs, and 94 ADs). MCI and AD patients were recruited from the Memory Clinic at the Jewish General Hospital (Montréal), whereas NECs were recruited from our laboratory database. Results showed that the number and composition of the factors varied across the groups. In the NEC group no well-defined factor was extracted, suggesting that in this group each item is measuring a unique cognitive ability. In the MCI group two factors were extracted, one reflecting Visual-Construction Skills/Executive Functioning (trail-making, cube-drawing, clock-drawing), and a second reflecting Working Memory (serial 7s, sentences), which was defined by only two poorly correlated items. In the AD group, five well-defined factors were extracted. These included Semantic/Working Memory (naming, serial 7s, orientation), Inattention (digit span, letter-taping, sentence repetition), Visual-motor Skills (trail-making, cube-drawing), and Executive Functioning (clock-drawing, verbal fluency, abstraction), with delayed recall as a stand-alone item. These results suggest that as cognitive impairment becomes more severe, the items assessing related cognitive abilities begin to correlate with each other and create factors, indicating that the impairment in each domain becomes more global. This study demonstrates that the factor structure of the MoCA differs between NECs, MCI, and AD patients and thus these groups should not be considered as a whole when examining underlying cognitive abilities in test performance.
Iterative Partial Volume Correction Algorithm for High Resolution PET

Funck, T., Zepper, P., Evans, A.C. and Thiel, A.

A new method has been devised for correcting partial volume effects (PVE) in PET by using structural information from MRI in conjunction with an iterative algorithm to correct the blurred PET image. Method An analysis pipeline known as CIVET produces a 3D mesh of interconnecting vertices that models the geometry of the cortical surface and determines the thickness of the surface at each vertex (Kim et al., 2005). Using the thickness of the cortex, it is possible to calculate the proportion of the PET signal that has “spilled out” from the the surface and thus to partially restore the true PET signal. Further restoring the original image is a difficult problem to solve analytically because the spatial distribution of the 3D blur has been collapsed into 2D. In order to overcome this obstacle, an iterative algorithm has been introduced. The purpose of this iterative algorithm is to estimate the original, unblurred image which must have given rise to the observed, blurred image. The algorithm makes an initial guess of the true, unblurred image using the partially restored vertices produced by an initial thickness-based partial volume correction (PVC). The test image is then blurred and divided by the actual observed image to produce an array representing the discrepancy between the test image and the observed image. The hypothetical guess of the original image is then updated by dividing by the array of residuals. The process continues until the guess of the original image produces a blurred image that is similar enough to the actual observed image to satisfy a given error tolerance, or until the maximum number of iterations has been reached. The iterative algorithm has been tested on simulated images of 5 x 5 x 5, with randomly generated values along a surface-like structure of varying thickness. The algorithm was run with a maximum number of 1000 iterations and an error tolerance of 0.001. 20 images were randomly generated and corrected for ten different levels of noise-to-signal ratios. Results The iterative algorithm is accurate on simple simulated images, even in the presence of significant noise. At a signal-to-noise level of .019 the error-to-signal ratio was 0.003 +/- 0.0003 and increased to an error-to-signal level of 0.169 at a noise-to-signal ratio of 0.0966 +/- 0.0116. Conclusion The use of both the thickness-based PVC and the iterative algorithm work well on theoretical data and now requires validation against real empirical data.
Link-N Peptide: A Potential Agent for Biological Repair of Human Intervertebral Disc

Gawri, R., Antoniou, J., Ouellet, J., Steffen, T., Roughley, P.J., Haglund, L. and Mwale, F.

Back pain is a fairly common problem which affects a large portion of the population across all ages and has an impact on quality of life. Intervertebral disc degeneration is the single most common implicated cause of back pain. Presently there is no medical treatment or therapeutic agent to address this problem and surgery is the only offered option. Link-N peptide represents the 16 amino acid sequence from the N-terminus of the link protein that stabilizes the proteoglycan aggregates present in cartilage and disc. Link-N peptide is released from the link protein as a result of proteolysis, and has been suggested to play a role in matrix homeostasis by promoting new matrix synthesis. We evaluated its regenerative potential in intact human intervertebral discs.

Materials and Methods: Lumbar IVDs were obtained through organ donations via Transplant Quebec. Discs from 7 individuals, 5 discs per spine, were harvested. Cells were isolated from nucleus pulposus (NP) and inner annulus fibrosus (iAF) regions of the discs. Single cells were beaded in 1.2% alginate and cultured in DMEM containing 1g/L glucose and 10% FBS. Alginate beads were exposed to 10-10000ng/ml Link-N peptide for 48 hours. Intact discs were prepared for organ culture by parallel cuts through the adjacent vertebral bodies close to the end plates, and the remaining bone and the calcified part of the cartilage endplates were removed using a high-speed bone burr. Discs were maintained and cultured with no external load applied in DMEM containing 1g/L glucose and supplemented with 1% FBS. Link-N was conjugated with 5-TAMRA dye then injected into the center of the disc. The distribution of Link-N in the medium and within the disc was studied to determine whether Link-N is retained in the disc. Discs from adjacent levels were matched for the degree of degeneration and were injected in their NP region with 50μCi 35SO4 along with 0.1mg or 1mg of Link-N in 100μl of medium per disc and harvested after 48 hours. Sustained effect of Link-N was evaluated by injecting the disc with Link-N and injecting 35SO4 one week later. Proteoglycan synthesis was evaluated by measuring 35SO4 incorporation

Results: When human lumbar disc cells from NP and iAF regions beaded in alginate were exposed to Link-N peptide for 48 hours, proteoglycan synthesis was observed to increase in a dose dependent manner with the maximal response at 1000ng/ml Link-N. (ABSTRACT TRUNCATED AT 400 WORDS).
What are the key factors and processes explaining the inconsistent results of the case management model efficacy for community-dwelling patients with cognitive impairment? A protocol of systematic mixed studies review

Khanassov, V., Vedel, I., Pluye, P. and Bergman, H.

Globally, the number of people affected by dementia rises exponentially as the population ages. Every four seconds a new case of dementia is detected around the world, equivalent to 7.7 million new cases every year. A consistent and widespread criticism of the care given to people with dementia and their caregivers in many countries, is that it is fragmented, rarely person-centered, suboptimal in terms of care quality and service use. To address the problem the case management model has emerged over the past decades which represents a collaborative process of needs assessment, care planning based on evidence-based care, collaboration, and advocacy for services to meet an individual’s health needs. Case management utilises a care manager who coordinates individual care with available resources, and may liaise with primary care physicians and specialised secondary care teams. Several studies conducted in USA and Europe have demonstrated varied results of the case management model including clinical outcomes (eg. cognition, mood, activities of daily living) and service use (eg. hospitalisation rate and nursing home placement). The conclusions highlight the importance of determining possible key elements that could explain these inconsistent results. Objective: to determine the role of various factors (patient characteristics, methodological features of studies) and processes (key structural features of interventions, primary care particularities) in clinical outcomes and service use for community dwelling patients with cognitive impairment. Method: The systematic mixed studies review method will be used which includes qualitative, quantitative, and mixed methods studies. Publications in English and French about case management, case coordination or care management of patient with cognitive impairment, dementia or Alzheimer’s disease dwelling in the community will be searched for using MEDLINE, Embase, PsycINFO between 1995 and 2012. A two-step process will be applied proceeding from titles to abstracts and then to the full articles by two independent reviewers. Differences in the coding will be resolved through consensus with the third reviewer. The search process will be conducted according to PRISMA guidelines. The 2010 revised CONSORT statement and the Mixed Methods Appraisal Tool (MMAT) will be used as a basis for assessing the reporting quality of qualitative, quantitative, and mixed methods studies. Expected results: The results of systematic review will be used by the Ministère de la Santé et des Services sociaux which is implementing the Alzheimer Plan for Quebec focused on primary care.
Investigation of Mouse Telomerase and Regulation by Associated Proteins

Kwan, R., Shawi, M., Zhu, S. and Autexier, C.

Telomerase is an RNA-dependent DNA polymerase that serves as a tumour marker found in 85% of all human cancer malignancies. The enzyme’s activity allows for immortalization of cancer cells by preventing their telomeres from progressive shortening, whereas normal somatic cells and tissues lack telomerase and will consequently undergo telomere attrition resulting in cell senescence or cell death. The catalytic subunit of telomerase, telomerase reverse transcriptase (TERT), is associated with proteins that modulate telomere maintenance and ultimately cell survival. Thus far, mouse models have been used to study telomerase within the context of a whole organism. However, contrary to humans, there have been very few mouse TERT (mTERT)-associated proteins that have been identified and characterized, and analysis of these mTERT-associated proteins has demonstrated no effects on telomerase activity or telomere length in vivo. Therefore, further investigation of mTERT-associated proteins is required to establish a detailed understanding of the species-specific differences between mouse and human telomerases and telomeres. Moreover, understanding telomerase and telomere regulation in mouse and human cells may inform us regarding the differential response to telomerase inhibition and telomere dysfunction that has been observed between human and mouse cells. A better understanding of the species-specific differences will be necessary to develop and validate therapeutic strategies that target telomerase through the use of rodent models. We hypothesize that there are currently undefined mTERT-associated proteins that can regulate the catalytic function of telomerase and telomere function. Our objective is to identify mTERT-associated proteins by means of affinity purification and mass spectrometry, and select candidates will subsequently be characterized by downregulating or overexpression to determine their effects on telomerase and telomere function. We have evaluated the efficacy of four different methods of mTERT purification: immunoprecipitation against streptavidin binding peptide (SBP), tandem affinity purification (TAP) using Protein G and SBP (GS), SBP purification, and FLAG immunoprecipitation. Samples from SBP purification show indications of optimal purification and have been subjected to mass spectrometry. We will discuss our progress regarding the identification and validation of mTERT-associated proteins.
A novel Endoplasmic Reticulum Stress Response Element regulating Prion protein also induces expression of other genes

Misiewicz, M., Jodoin, J., Ruths, D. and LeBlanc, A.C.

The exact function and role of the prion protein (PrP), despite many years of research, remains elusive. In order to help better understand the cellular processes PrP plays a role in, we investigated a novel promoter element identified in the prion protein gene (PRNP) promoter. Originally discovered by homology to the endoplasmic reticulum stress response element (ERSE), this novel element (called ERSE-like 26) closely resembles the canonical ERSE, CCAAT-N9-CCACG, a transcription factor binding site which is sufficient to up-regulate gene expression of a number of well-known ER stress response genes. The goal of this study was to identify genes containing this novel element and characterize the degree of their co-regulation with PRNP. Methods – We first conducted a search of the entire human genome for genes with the ERSE-like 26. Next, we treated cultured primary human neurons with the pharmacological ER stressors Brefeldin A, Tunicamycin and Thapsigargin, and conducted Reverse Transcriptase PCR (RT-PCR). ERSE-like 26 genes were also tested in human breast cancer carcinoma MCF-7 cells, using the drugs. Next, to identify the transcription factors involved in ERSE-like 26 activity, we produced a reporter construct containing a secreted luciferase gene and the 100bp fragment of the PRNP promoter containing the ERSE-like 26 element. This construct was co-transfected into HEK-293 cells with plasmids encoding the activated transcription factors XBP-1 or ATF6a. We next transfected MCF-7 cells with the identified responsible factor XBP-1 to reproduce the effects of pharmacological perturbation on ERSE-like 26 containing genes. Finally, a literature search and clustering of gene function was conducted, in order to gain a broader understanding of the cellular context of ERSE-like 26 genes. Results - The bioinformatic analysis revealed the ERSE-like 26 sequence in the promoter of 38 genes. Of these 38 genes, 20 were detected by RT-PCR in treated human neurons and all of them showed up-regulation by at least one of the drugs in at least one genetically unique neuron preparation. Eighteen genes were found in MCF-7 cells, and showed similar results. The luciferase reporter experiments showed that activated XBP-1, but not ATF6a induced activity of the ERSE-like 26 element in HEK-293 cells. Finally, transfection of XBP-1 into MCF-7 cells showed up-regulation for some ERSE-like 26 genes. Clustering the 38 genes based on function revealed the up-regulation for genes involved in the production of GABA, mitochondrial function oxidative stress, and cell adhesion, all potential novel roles for PrP.
Evaluation of a novel Caspase-6 inhibitor as a potential treatment for Alzheimer Disease

Pakavathkumar, P., Ahlfors, J.E. and LeBlanc, A.C.

Caspase-6 activity is found abundantly in the neuropil threads, neuritic plaques and neurofibrillary tangles of familial and sporadic forms of Alzheimer disease. Caspase-6 induces axonal degeneration and memory impairment in mice (abstract submitted at this meeting). Therefore, inhibiting Caspase-6 represents a potential treatment for Alzheimer disease. Unfortunately, there are no known natural inhibitors of Caspase-6. In this study, we investigated a newly developed irreversible Caspase-6 inhibitor called NWL-117 developed by New World Laboratories. Objective: Determine if the NWL-117 compound can be used as a potent, specific, and non-toxic inhibitor of active Caspase-6. Methods: The toxicity of the Caspase-6 (Casp6) inhibitor, NWL-117, was verified on the HCT116 cell line and human primary neurons by MTT, propidium iodide FACS analyses, and Caspase-3 processing by western blots or activity by fluorogenic assays. Dose-dependent inhibition of Casp6 was assessed by in vitro fluorogenic assays with purified recombinant active Casp6 and on HCT116 cells transfected with a self-activated form of Casp6. Inhibition of active Casp6 was also assessed in primary human neurons in culture. Casp6 activity in cellulo was assessed with FLICATM-Casp6 assays. Specificity of the NWL-117 inhibitor for Casp6 was investigated by conducting dose response curves on other purified recombinant caspases by fluorogenic assays. Results: The NWL-117 was not toxic to the HCT116 cells or the neurons at 20 to 100 µM concentrations. In vitro, a dose dependent inhibition of recombinant active Casp6 was observed between 50 nM (50%) and 5 µM (100%). NWL-117 was more potent than the peptide inhibitor, Ac-VEID-fmk. A 1 µM concentration of NWL-117 inhibited almost 50% of active Casp6 in HCT116 cells and showed a dose-dependent inhibition between 5 µM and 100 µM concentrations. Western blot analyses showed that the Casp6 was processed into its active form in the absence or presence of the inhibitor. Conclusions: These results show that NWL-117 is cell permeable and non-toxic at high concentrations. NWL-117 is a potent inhibitor of the processed active form of Casp6. Therefore, the NWL-117 can be used to assess if Casp6-mediated axonal degeneration can be inhibited and possibly reversed in primary human neurons and mouse brains. If so, this compound is an interesting “lead” compound to develop as an inhibitor of Casp6 in Alzheimer disease patients.
ERP Measures of Working Memory in Patients with Mild Cognitive Impairment, Alzheimer Disease, and Healthy Controls


Many older adults diagnosed with mild cognitive impairment (MCI) are thought to be in a preclinical stage of Alzheimer disease. Working memory (WM) is known to be impaired in both MCI and early Alzheimer disease. We examined the sensitivity of the P300 event-related brain potential (ERP) to working memory function in 16 Alzheimer disease (AD) patients, 21 MCI patients, and 27 age- and education-matched normal elderly controls (NECs), using a verbal n-back task with 0-, 1-, and 2-back loads. AD patients performed significantly less accurately than both MCI and NEC groups at the 1- and 2-back loads and MCI patients performed worse than NECs during the 2-back load. With respect to P300 amplitude, there was a Group X WM Load interaction, such that P300 amplitude decreased with Load (NEC: 0-back > 2-back; MCI: 1-back > 2-back; AD: 1-back > 2-back). Interestingly, the NECs showed significantly larger P300 amplitudes than the MCI and AD patients at the 2-back load, while the patient groups did not differ from each other. That is, although the MCI patients showed better behavioural performance than the ADs at the 2-back load, the P300 amplitude was equally reduced in both groups, suggesting that maintaining performance at this WM load was particularly taxing for the MCI group. Overall, the results indicate that changes in resource allocation may be a sensitive indicator of working memory impairment in pre-clinical AD.
Effects of Caspase-6 gene SNPs in Alzheimer disease

Putorti, M.L. and LeBlanc, A.C.

Caspase-6 (Casp6) is a cysteine protease activated in brains affected by Alzheimer disease (AD) and induces axonal degeneration rather than cell death. There are nine single nucleotide polymorphisms (SNPs) in the CASP6 coding region sequence. Six of them generate polymorphic Casp6 proteins (Alanine34Glutamate, Glutamate35Lysine, Arginine65Tryptophan, Glycine66Arginine, Alanine109Threonine and Threonine182Serine) and three of them are synonymous SNPs (Alanine111Alanine, Glutamate135Glutamate and Isoleucine160Isoleucine). Our goal focuses on investigating the impact that CASP6 SNPs have on Casp6 activity in AD. In order to achieve our goal, pCEP4-CASP6 mammalian expression vectors have been constructed with missense or synonymous SNPs by polymerase chain reaction (PCR) directed mutagenesis. Vectors have been amplified in DH5α bacteria. Human colon carcinoma HCT116 cells have been transfected with these vectors using polyethylenimine (PEI). Casp6 expression and processing (activation) have been assessed by Western blot and Casp6 activity fluorogenic assays using the N-Acetyl-Valine-Glutamate-Isoleucine-Aspartate-7-Amino-4-Trifluromethyl Coumarin (Ac-VEID-AFC) substrate. Four SNPs show decreased Casp6 activity (40 % to 60 % decrease, despite normal expression). Also, pET23b-Casp6-His bacterial expression vectors have been constructed with missense SNPs. Recombinant Casp6 proteins have been generated in BL21 DE(3) pLys bacteria and the histidine tag has facilitated the protein purification on a nickel column using the fast protein liquid chromatography (FPLC) system. Casp6 expression and processing (activation) have been measured by Western blot and by Casp6 activity fluorogenic assays. As in cellulo, three SNPs show again a decrease in Casp6 activity. Potential discovery of a SNP more frequently found in individuals affected by AD compared to non-AD control individuals could lead to a diagnostic test.
Objective: Caspase-6 activity is found abundantly in the neuropil threads, neuritic plaques and neurofibrillary tangles of familial and sporadic forms of Alzheimer disease (AD). Interestingly, some non-cognitively impaired aged individuals show the presence of abundant active Caspase-6 in the entorhinal cortex. A study showed that aged individuals with active Caspase-6 in the entorhinal cortex had lower cognitive scores than those that did not have active Caspase-6 in their brains. These results suggested that Caspase-6 activity might be used as an early biomarker for AD. In this study, we evaluated the levels of Tau cleaved by Caspase-6 (Tau\(^{\text{Casp6}}\)) as a possible biomarker of AD. Methods: An ELISA was set up against Tau full length and Tau\(^{\text{Casp6}}\) and optimized to quantitatively detect Tau\(^{\text{Casp6}}\) in pg/mL amounts. The presence of Tau\(^{\text{Casp6}}\) was tested in post-mortem cerebrospinal fluid (CSF) of non-cognitively impaired, mild-cognitively impaired and AD individuals. The amount was compared to the levels of Tau\(^{\text{Casp6}}\) observed in the hippocampus (CA1, CA2, CA3 and CA4), subiculum, and entorhinal, trans-entorhinal and temporal cortices by immunohistochemistry using an anti-Tau\(^{\text{Casp6}}\) specific antiserum. The levels of Tau\(^{\text{Casp6}}\) in CSF were compared to the global cognitive, mini-mental state examination, Braak stage, working, episodic, semantic memory as well as perceptual speed and visuospatial ability scores of these individuals. Correlations between levels of CSF Tau\(^{\text{Casp6}}\) and cognitive or pathological scores were assessed using Spearman Rank Correlation. Results: Tau\(^{\text{Casp6}}\) levels were significantly higher in individuals with AD compared to those with non-cognitive impairment. The Tau\(^{\text{Casp6}}\) levels in the CSF correlated significantly and positively with levels of Tau\(^{\text{Casp6}}\) detected in the temporal cortex, entorhinal cortex, subiculum, and hippocampus (CA1, CA3 and CA4). The Tau\(^{\text{Casp6}}\) levels correlated significantly and negatively with the global cognitive, mini-mental state examination, and episodic, working, and semantic memory scores of these individuals. Comparisons between Tau\(^{\text{Casp6}}\) levels in the CSF did not correlate with perceptual speed or visuospatial ability scores. Conclusions: We conclude from these results that the levels of Tau\(^{\text{Casp6}}\) in the CSF correlate positively with the severity of dementia in aged individuals. This work provides a proof of principle to assess Tau\(^{\text{Casp6}}\) levels in pre-mortem CSF. Further work is required to determine the specificity of Tau\(^{\text{Casp6}}\) for AD by assessing CSF Tau\(^{\text{Casp6}}\) levels in other neurodegenerative diseases.
Impact of Ser43 phosphorylation on biophysical properties of familial prion protein mutants

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In familial prion-related diseases (PrPD), conformational conversion of a mutated soluble cellular prion protein (PrPc) into an insoluble and partially protease-resistant isoform (PrPRes) occurs and allows propagation of transmissible spongiform encephalopathies. The physiological events involved in conformational PrPc conversion into PrPRes are not well-known. Our laboratory has previously shown that phosphorylation of PrPc at Ser43 by Cyclin-dependant kinase 5 (Cdk5) is (1) converted in vitro as PrPRes fibrils and aggregates and (2) detected in vivo in scrapie-infected mice brains. We want to study the role of Cdk5-mediated phosphorylation in familial PrPD, and more particularly the impact of this phosphorylation on biophysical properties of familial PrP mutants (fPrPms). For this purpose, we have selected different fPrPms associated with one of the three major familial PrPD and each located in different prion domains (Y145Stop, A117V, D178N, T188A, E200K and the V210I) and harboring the corresponding Met or Val residues at the polymorphic codon 129. Firstly, kinetic curves of fPrPm phosphorylation by Cdk5, established with in vitro kinase assays, show that all these fPrPm can be phosphorylated to the same degree as PrPWT and the nature of residue 129 does not influence its extent. Secondly, to study the impact of phosphorylation at Ser43 on propensity of fPrPm to aggregate or form fibrils, we developed a novel approach, based on Thioflavin T binding to amyloid structures, to monitor aggregation kinetics upon phosphorylation. This approach coined IKTA (In vitro Kinase reaction coupled to Thioflavin T binding Assay) revealed that the Cdk5-phosphorylated PrPWT form with a Met codon at position 129 (129Met) fibrillarized more rapidly than the PrPWT(129Val). Furthermore, all fPrPm tested with the exception of fPrPE200K, showed an increase in aggregation rate in comparison to their respective PrPWT form. A quantitative analysis following PK digestion will demonstrate whether these mutants exhibit different resistance profiles relative to their respective PrPWT forms. The results show that phosphorylated familial PrP autosomal dominant mutants have an increased propensity to form amyloid structures, indicating that prion phosphorylation at this site has a significant impact on fPrPm conformation and capacity for amyloid formation. This study, in conjunction with an in vivo study, will provide a better understanding of the influence of familial prion mutations on PrPc conversion induced by post-translational modification in the pathological context of familial PrPD. Furthermore, inhibition of prion phosphorylation in familial PrP mutants carriers may serve as a feasible therapeutic approach to prevent prion mediated neurodegeneration.
Interferon inducible transmembrane (IFITM) proteins are a small protein family. They were recently shown to inhibit a number of viruses including influenza A viruses, flaviviruses, filoviruses, SARS coronaviruses, vesicular stomatitis virus and HIV-1. This indicates the important role of IFITMs in the interferon-mediated host antiviral defense. Our group previously reported that IFITM1, IFITM2 and IFITM3 inhibited HIV-1 replication in the SupT1 CD4+ T cells. In order to understand the mechanisms behind the anti-HIV-1 activity of IFITM proteins, we grew HIV-1 in IFITM1-expressing SupT1 cells over a long period of time and obtained an HIV-1 revertant that exhibited wild-type replication capacity in the presence of IFITM1. Sequencing the entire viral genome revealed four mutations, including Vpu36, EnvR311K, EnvG367E and Envg7823a. Further mutagenesis studies showed that the Vpu36 and EnvG367E mutations together were sufficient to confer resistance to IFITM1. In contrast to the parental virus, the revertant virus was resistant to the inhibition by soluble CD4 (sCD4) and the broadly neutralizing antibody VRC03 that targets the CD4 binding site on gp120, which suggests that the revertant virus has become CD4-independent. Collectively, our work indicates that IFITM1 poses a strong inhibitory pressure on HIV-1 replication, which drives the virus to evolve and escape from IFITM1 restriction.
Lack of evidence for the TAR-TAR kissing but a dominant role of TAR bulge in HIV-1 genomic RNA dimerization

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The hallmark of retroviruses is the packaging of two identical copies of unspliced viral RNA that are noncovalently linked near their 5’ends. A dimeric genome appears essential for viral replication cycle since, among other effects, it facilitates gRNA strand transfer during reverse transcription. HIV-1 genomic RNA dimerization appears largely controlled by the first 500 nucleotides (nts) from the 5’ end of gRNA and by proteolytic processing of the Gag polyprotein Pr55gag. The 5' untranslated region of HIV-1 genomic RNA (gRNA) contains 2 stem-loops that appear equally important for gRNA dimerization: the 57-nt long 5' TAR, at the very 5’ end, and the 35-nt long SL1 (nt 243-277). At least some level of dimerization is thought to be controlled by the dimerization initiation site (DIS), a 6-nt long palindrome which is located in the apical loop of SL1. Although the role of TAR in HIV-1 gRNA dimerization has been shown, the mechanism of its action is not understood. The TAR-TAR kissing hypothesis, supported by the presence of a phylogenetically conserved palindrome (termed palindrome 2 here) located in the apical loop of TAR, has been proposed as a possible mechanism for HIV-1 gRNA dimerization. Here, we investigated the TAR-TAR kissing hypothesis by severely destabilizing the kissing potential of palindrome 2 and reconstituting the kissing potential by introducing compensatory mutations that form an alternative palindrome with a duplex stability similar to that of wild type. The impact of mutations on HIV-1 gRNA dimerization was studied in the context of WT, DIS-inactivated, and protease-inactive HIV-1HXB2. There is another palindrome sequence, termed palindrome 1 here, which consists of nucleotides 18- CAGAUUG. We also tested the TAR-TAR kissing hypothesis involving palindrome 1 sequence using the same strategy as the one for palindrome 2 study. Mutations destabilizing potential duplex formation in both palindromes reduced gRNA dimerization which is consistent with the kissing-loop hypothesis. However, the dimerization defects were not corrected in compensatory mutations which is inconsistent with the kissing hypothesis. Notably, we observed an unexpected defect in HIV-1 gRNA dimerization when UCU bulge was deleted. Based on these results we conclude that TAR is very important for gRNA dimerization, but plays its role not via TAR-TAR kissing and we conclude that deleting the TAR bulge impairs dimerization by disturbing an interaction of TAR with non-TAR and non-SL1 gRNA sequences, or by misdirecting the viral or cellular proteins that are affected by these TAR mutations.
Development of small RNA inhibitors of HIV

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Nucleic acid therapies targeting HIV replication have the potential to be used in conjunction with, or in place of the standard combination therapies. Improvements in the delivery of nucleic acids to HIV target cells could lead to the eventual use of these agents in drug therapy, and several studies have supported the possibility of using them in a gene therapy setting. Among the different classes of nucleic acid therapies, several hammerhead and hairpin ribozymes (Rzs, RNA enzymes) have been designed to target HIV RNA, and some have advanced to clinical trials. Results have demonstrated that Rz therapy is safe, however improvements in efficacy are required. In an attempt to improve upon the stability and activity of available Rzs targeting HIV RNA, we have designed and evaluated several Specific On/off Adaptor, Hepatitis Delta Virus (SOFA-HDV) Rzs. We have developed an algorithm to identify SOFA-HDV Rzs that have the ability to target the majority of HIV clinical isolates published on the Los Alamos HIV database. Rzs were evaluated for their ability to cleave HIV RNA in vitro and for their ability to restrict HIV virus production in cell culture. We were able to identify one SOFA-HDV Rz with a potent effect on HIV production comparable to other small RNA molecules, which have recently entered clinical trials. In addition, we have shown that the target site of this Rz is also accessible to a short hairpin RNA, and that our SOFA-HDV Rz is active against different HIV strains. Our results suggest that the target site for SOFA-HDV Rzs needs to be accessible, highlighting the importance of target site selection in the design of Rzs targeting HIV RNA. We have identified a Rz that has a high potential to be used effectively in a combination gene therapy approach for the treatment of HIV infection, and may be able to be used in drug therapy with appropriate delivery vehicles.
HIV-1 transcription is increased by the RNAi pathway proteins TRBP and PACT

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The cellular proteins TRBP (TAR-RNA Binding Protein) and PACT (PKR-Activator) are two RNA binding proteins with 40% homology. They both bind to PKR but while TRBP inhibits PKR activity, and enhances translation in cell, PACT enhances PKR activity and inhibits translation. In addition, TRBP is an integral part of the RNA-induced silencing complex (RISC) which mediates RNA interference (RNAi) and PACT has been found to be associated with the RISC and to increase RNAi activity. In HIV-1 infected cells, TRBP is an essential protein for viral replication and surprisingly, PACT has recently been shown to also enhance expression of viral genes. To get further insight into the mechanism of HIV-1 gene expression, we examined the effect of TRBP and PACT on the RNA expression of reporter genes that are under the control of HIV-1 LTR and SV-40 viral promoters. In addition to the known translational activity of TRBP and PACT, we identified that these proteins also increase RNA levels produced from these viral promoters. To characterize the transcriptional activation domain of TRBP and PACT, we expressed different fragments of the proteins fused to a DNA binding domain and tested their ability to activate transcription in yeast and in mammalian cells. In these assays, we mapped their transcriptional component to a 26aa C-terminal fragment of both proteins. Finally, in the context of HIV infected cells, we show that siRNAs against TRBP decrease the production of HIV RNA. This work describes a surprising regulation of HIV transcription by TRBP and PACT, and suggests an intriguing new mechanism by which these RNA-binding proteins increase viral RNA production. It opens the way to interconnections between viral transcription, RNAi and PKR regulation.
Role of the double-stranded RNA binding domains of RNA helicase A in the binding to RNA

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RNA helicase A (RHA), a member of the DExH subclass of RNA helicases, is involved in multiple steps of RNA metabolism during HIV-1 replication. RHA is unique among the superfamily 2 RNA helicases in containing two double-stranded RNA binding domains (dsRBD1 and 2) at the N-terminus, and the role of these two domains in facilitating RHA binding to RNA and unwinding of duplex RNA is examined herein. We found that RHA preferentially binds double-stranded RNA or highly structured region of HIV-1 RNA. Mutational analysis demonstrates that two dsRBDs are responsible for this RNA binding property in a cooperative fashion.
THE POTENTIAL ROLE OF GAS6 IN CANCER-INDUCED VENOUS THROMBOSIS

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Rational: Venous thrombosis is the most common morbid complication related to cancer and its treatments. Underlying mechanisms of this disease are not well understood, mainly because animal models studying this disease are scarce and in most cases very crude. Growth arrest specific 6 (gas6) is a vitamin K dependent secreted protein that is expressed in different cell types. Gas6 is overexpressed in certain cancers, and in regards to thrombosis, gas6 null mice do not develop thrombi as readily as wild type mice do, we have also showed in our lab that Gas6 levels are increased in plasma of patients with recurrent venous thrombosis, which is a sign of a present tumor.

Objective: To elucidate the role of Gas6 in cancer-induced venous thrombosis in mice.

Methods: We injected wild type and gas6 null C57Bl6 mice with M27 murine lung cancer cells. Mice were allowed to develop end stage cancer. We then induced venous thrombosis by injuring their inferior vena cava with Ferric Chloride, and monitored thrombus formation in these mice over time using an ultrasonography method previously developed in the lab. Also, to look at the mechanism of cancer induced thrombosis, we performed a series of co-culture experiments and cultured endothelial cells isolated from the wild type and Gas6 null mice, in the presence of M27 cells. We then performed a whole genome microarray analysis on these samples to identify differential genes expression in the presence of cancer by the lack of gas6.

Results: We show that in the wild type background, end stage cancer causes an increase in thrombus size where as gas6 null mice are protected against cancer induced venous thrombosis. Microarray analysis determined 28 genes of interest differentially expressed in the presence of cancer in the two genotypes. Based on the statistical analysis the genes for Thrombospondin 2 and C1 inhibitor are the most prevalent genes, further analysis is still required to verify the role of these genes in cancer induced thrombosis with regards to gas6.

Conclusion: These results suggest that gas6 may have a pathophysiologic role in cancer-induced thrombosis, possibly by modulating certain genes in the endothelium.
Erythropoietin-Induced Hypertension and Vascular Injury in Mice Overexpressing Human Endothelin-1 was Attenuated By Exercise

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**Background:** Erythropoietin (EPO) is used to correct anemia in chronic kidney disease (CKD). EPO has been shown to increase blood pressure (BP) in patients and experimental animals with CKD. This BP rise can be blunted by endothelin (ET) A receptor blockers. Chronic exercise prevents or reduces development of cardiovascular disease. However, it is unknown whether exercise prevents EPO-induced hypertension. We hypothesized that EPO treatment would exacerbate endothelin (ET)-1-induced vascular damage and increase BP, and that exercise training might prevent these effects.

**Design and methods:** Eight to ten-week old male mice overexpressing human preproET-1 in the endothelium (eET-1) were treated with EPO (100 U/kg, s.c, 3 times/week) or not (control), and subjected to swimming exercise (1 h/d, 5 d/week) for 8 weeks or maintained in sedentary condition (n=7-8). Systolic BP (SBP) was measured by the tail-cuff method. Endothelial function was assessed in mesenteric arteries by pressurized myography. In aorta, we assessed NADPH oxidase activity by lucigenin chemiluminescence, reactive oxygen species (ROS) by dihydroethidium staining and VCAM-1 and monocyte/macrophage infiltration by immunofluorescence staining.

**Results:** EPO increased SBP by 24 mmHg ($P<0.05$) and impaired vasodilatory responses to acetylcholine (68% vs. 91%, $P<0.01$) compared to controls. EPO increased NADPH oxidase activity 1.5-fold ($P<0.01$) and ROS production in aorta compared to controls. EPO increased monocyte/macrophage infiltration (2.6-fold, $P<0.01$) and tended to increase VCAM-1 expression in aorta compared to controls. All of the above were prevented by exercise ($P<0.05$).

**Conclusions:** Exercise prevents EPO-induced BP elevation, endothelial dysfunction and vascular oxidative stress and inflammation in eET-1 mice.
Inhibition of Four-and-a-half Lim domain protein 2 increases survival and migratory capacity of human early outgrowth endothelial progenitor cells through upregulation of sphingosin kinase-1: Implications for endothelial regeneration

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Inhibition of Four-and-a-half LIM domain protein-2 (FHL-2) attenuates atherosclerotic lesion formation and increases endothelial cell migration. Endothelial progenitor cells (EPCs) substantially contribute to endothelial repair. We investigated the role of FHL-2 in the regulation of early outgrowth EPC number and function. Methods and Results: Early outgrowth EPCs were obtained from human peripheral blood. FHL-2 knockdown in EPCs by small-interfering RNA (siRNA) resulted in a significant increase in EPC number and a reduction of apoptosis (by 40%), as indicated by a decrease of cleaved caspase-3, through activation and translocation to the membrane, of sphingosine kinase-1 (SK-1), enzyme that metabolizes sphingosine-1 phosphate (s1p). Furthermore, FHL-2 siRNA increased significantly (2 fold) stromal derived factor (SDF) -1- induced EPC migration; through upregulation of a-v/ß-3 and a-v/ß-5 integrins; this was associated with an increase of the F-actin binding protein cortactin, known to promote migration. Interestingly, increased SDF-1- induced EPC migration and upregulation of cortactin by FHL-2 siRNA were totally prevented by CAY10621, a specific inhibitor of SK-1. In addition stimulation of EPCs with exogenous s1p peptide significantly decreased apoptosis and increased SDF-1- induced migration. These results were confirmed in vivo using FHL-2 knockout (FHL-2 -/-) mice. Moreover, apoptosis was significantly decreased and migration increased in endothelial cells exposed to the conditioned medium of FHL-2 -/- vs. WT EPCs. These effects were abolished by VPC23019, an antagonist of sphingosine-1 phosphate receptor-1 and 3. Finally, reendothelialization after focal carotid endothelial electric injury in WT mice was significantly increased after application of spleen-derived endothelial progenitor cells from FHL-2 -/- mice vs. WT mice. Conclusions: Our findings suggest that FHL-2 negatively regulates early outgrowth EPC function and secretion of paracrine factors. FHL-2 inhibition reduces apoptosis, enhances survival and migratory capacity of EPCs and ECs by upregulating SK-1/s1p pathway, integrin subunits and cortactin; which results in the improvement of endothelial regeneration.
Red blood cells (RBC) are produced at a rate of 2.3 x 10^6 cells per second by a dynamic and exquisitely regulated process known as erythropoiesis. During this development, RBC precursors synthesize the highest amounts of total organismal heme (75-80%), which is a complex of iron with protoporphyrin IX. Heme is essential for the function of all aerobic cells, but if left unbound to protein, it can promote free radical formation and peroxidation reactions leading to cell damage and tissue injury. Therefore, in order to prevent the accumulation of ‘free’ heme, it is imperative that cells maintain a balance of heme biosynthesis and catabolism. Physiologically, the only enzyme capable of degrading heme are heme oxygenase 1 & 2 (HO). Red blood cells contain the majority of heme destined for catabolism; this process takes place in splenic and hepatic macrophages following erythrophagocytosis of senescent RBC. Heme oxygenase, in particular its heme-inducible isoform HO1, has been extensively studied in hepatocytes and many other non-erythroid cells. In contrast, virtually nothing is known about the expression of HO1 in developing RBC. Likewise, it is unknown whether HO1 plays any role in erythroid cell development under physiological or pathophysiological conditions. Using primary erythroid cells isolated from mouse fetal livers (FL), we have shown that HO1 mRNA and protein are expressed in undifferentiated FL cells and that its levels, somewhat surprisingly, increase during erythropoietin-induced erythroid differentiation. This increase in HO1 can be prevented by succinylacetone (SA), an inhibitor of heme synthesis that blocks 5-aminolevulinic acid dehydratase, the second enzyme in the heme biosynthesis pathway. Moreover, we have found that down-regulation of HO1 via siRNA increases globin protein levels in DMSO-induced murine erythroleukemic (MEL) cells. Similarly, compared to wild type mice, FL cells isolated from HO1 knockout mice (FL/HO1-/-) exhibited increased globin and transferrin receptor levels and a decrease in ferritin levels when induced for differentiation with erythropoietin. Following induction, compared to wild type cells, FL/HO1-/- cells showed increased iron uptake and its incorporation into heme. We therefore conclude that the normal hemoglobinization rate appears to require HO1. On the other hand, MEL cells engineered to overexpress HO1 displayed reduced globin mRNA and protein levels when induced to differentiate. This finding suggests that HO1 could play a role in some pathophysiological conditions such as unbalanced globin synthesis in thalassemias.
Txnip (VDUP-1) as a novel regulator of erythroid differentiation

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Red blood cell pathologies are among the most prevalent causes of mortality and morbidity worldwide, highlighting the need to find novel targets to efficiently modulate erythropoiesis. Txnip, a member of the arrestin family, is an adaptor protein involved in multiple cellular processes, such as glucose metabolism, cell growth, cancer, cardiac homeostasis and inflammation. More recently, a role has emerged for Txnip in hematopoiesis, namely in the quiescence of hematopoietic stem cells and the differentiation of natural killer cells. In a cellular model, the mouse erythroleukemia (MEL) cell line, we observe a rapid and robust increase in Txnip levels upon treatment with chemical inducers of erythroid differentiation, followed by a marked decrease at later stages. Our studies indicate that its levels are regulated by the p38 MAPK, previously associated with red blood cell differentiation. In order to explore the implications of those results in vivo, we recently obtained Txnip-/- mice from the group of Dr. Simon Hui (UCLA). Preliminary results with knockout mice indicate increased splenic erythropoiesis and modified erythroid precursors distribution in the bone marrow. We are currently analysing the distribution of erythroid populations in a larger sample of Txnip knockout mice by flow cytometry of spleen and bone marrow cells, complemented by blood analysis of red cell parameters. In parallel, in vitro experiments, using knockdown and overexpression strategies will shed light on the mechanism by which Txnip levels influence erythropoiesis. The modulation of erythropoiesis by Txnip could have interesting clinical applications since known, although non-specific inducers of Txnip exist, such as HDAC inhibitors and demethylating agents, while inhibitors of Txnip could be generated.
Hemojuvelin (HJV) is a bone morphogenetic protein (BMP) co-receptor that plays a pivotal role in systemic iron homeostasis. Mutations in HJV are causatively linked to juvenile hemochromatosis, an early onset and severe form of hereditary iron overload. The marked suppression of hepcidin expression observed in juvenile hemochromatosis patients and in HJV-/- mice, suggests a function of hemojuvelin, as an upstream regulator of this liver-derived iron-regulatory peptide hormone. To elucidate the role of HJV in iron-sensing pathways, we employed HJV-/- mice and analyzed their responses to dietary or other iron manipulations. 8-weeks old HJV-/- and wild type (HJV+/+) control mice were placed in diets of varying iron content (low, normal and high) for 4 weeks. Additionally, a fourth group of mice was subjected to acute iron overload by intraperitoneal injection of iron dextran. Serum iron parameters, liver iron deposition and hepatic hepcidin, pSmad1/5/8 and BMP6 expression were assessed. Serum iron levels were significantly increased in HJV-/- mice in comparison to wild type controls in all diet regimens. Interestingly, transferrin was highly saturated (97%) in HJV-/- mice regardless the iron content of the diet. Massive liver iron deposition in HJV-/- mice was assessed with both a qualitative (Prussian blue stain) and quantitative (ferrozine assay) technique. As expected, hepatic levels of hepcidin mRNA correlated to dietary iron intake in wild type mice. Surprisingly, hepcidin mRNA expression was likewise responsive to dietary iron intake in HJV-/- mice, despite the marked overall suppression of hepcidin mRNA levels as compared to wild type controls. HJV-/- mice placed in low and normal iron diets exhibited significantly higher levels of BMP6 mRNA compared to wild type controls. Our data are consistent with earlier observations that the disruption of HJV leads to suppression of hepcidin expression and severe iron overload. Furthermore, our data suggest that HJV is not essential for iron sensing and rather acts as an enhancer for hepcidin expression. Importantly, our findings uncouple the iron-dependent regulation of hepcidin expression from alterations in transferrin saturation, at least in HJV-/- mice.
Arterial restenosis, which occurs in up to 20% of angioplasty patients, is characterized by an excessive vascular smooth muscle cell proliferation resulting from the removal of the endothelial cell lining. Circulating endothelial progenitor cells (EPCs) have the ability to re-colonize and repair the damaged vascular endothelium, reducing restenosis. Nitric oxide (NO) contributes to mobilization and functional activity of EPCs, and estrogen has been shown to increase circulating EPC levels and accelerate the reendothelialization process by EPCs in mice. Moreover NO is important for transducing estrogen-dependent signalling and reendothelialization. We hypothesized that overexpressing endothelial nitric oxide synthase (eNOS), or treatment with estrogen, would potentiate the beneficial effects of EPCs in the context of restenosis. We found that native human early outgrowth EPCs (hEPCs) did not have any effect on human coronary artery smooth muscle cell (HCASMC) proliferation and migration In vitro, evaluated by BrdU incorporation and wound scratch assay respectively. In contrast, the NO donor SNAP significantly decreased the proliferation and migration of HCASMCs. Thereafter, hEPCs were either transfected with a human eNOS plasmid or stimulated with 17β-estradiol (E2) prior to being co-cultured with HCASMCs. Total eNOS protein and eNOS phosphorylation levels were increased by 3- to 3.5-fold in eNOS-transfected or E2-stimulated hEPCs, evaluated by western blot. This was associated with a 3-fold increase in NO production, performed by DAF-FM diacetate immunofluorescence (p
HDAl's Impact Calcium Homeostasis in Cardiac Differentiated H9c2 Cells

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Heart failure is the leading cause of death in both men and women of Western countries. The pathophysiology of heart failure is associated with abnormalities in intracellular calcium control. Calsequestrin (CSQ2), a calcium storage protein in cardiomyocytes, is negatively regulated by the transcription factor Egr-1 thus altering calcium availability for cardiac contraction/relaxation. Here, we tested the hypothesis that the proteins complexed to Egr-1 and/or their post-translational modifications would affect regulation of CSQ2 expression. Egr-1 and Sp1 compete for binding at the CSQ2 promoter, but also bind one another. In fact, together they form a complex with another ubiquitous transcription factor YBX-1. This complex was identified in vivo and in vitro by a series of co-immunoprecipitations. To test the idea that complex formation and CSQ2 expression could be affected by acetylation, histone acetyltransferase (HAT) inhibitors and histone deacetylase (HDAC) inhibitors were used to respectively decrease or increase acetylation within cells. We found that acetylation did not impact the formation of the Egr-1: Sp1: YBX-1 complex thought to regulate CSQ2 expression. However, changes in CSQ2 expression were observed when acetylation was modified by HAT and HDAC inhibitors. We conclude that acetylation modifies CSQ2 expression although perhaps not by means of the Egr-1: Sp1: YBX-1 complex even though Egr-1 is known to be acetylated.
Involvement of Lipid Rafts and Caveolae in gas6-Axl Signaling in Endothelial Cells

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Lipid rafts are localized regions of plasma membranes that contain primarily cholesterol and glycosphingolipids. Many receptor tyrosine kinases including the epidermal growth factor receptor, the platelet derived growth factor receptor, and the insulin receptor, localize specifically to these structures. Lipid rafts have been shown to play a role in intracellular signaling events. More specifically, diverse processes such as dimer formation, autophosphorylation, and intracellular adaptor protein recruitment/activation have been specifically involved in receptor tyrosine kinase/lipid raft interactions. Caveolae, composed mainly by caveolin-1, are defined as a subclass of lipid rafts and form well-shape invaginations on the cell surface. Gas6 is a vitamin K dependent protein that is post-translationally modified by an enzymatic process called γ-carboxylation. Despite its structure, no role in the coagulation cascade has been shown for gas6. Interestingly, studies using gas6 null mice highlighted that gas6 may represent a potential target for anti-coagulant therapy as these mice are protected from lethal thromboembolism. However, gas6 action mechanism remains to be elucidated. Axl, the receptor for gas6, is a receptor tyrosine kinase whose association with lipid rafts and more specifically with caveolae has not been elucidated. We hypothesize that gas6/Axl mediated intracellular signaling is dependent on its localization to lipid rafts/caveolae in endothelial cells. First, we show that gas6-induced c-Src, Akt and ERK1/2 phosphorylation is mediated via Axl by using Axl siRNA in human umbilical vein endothelial cells (HUVECs). Then, lipid rafts were isolated from HUVECs by a detergent-free lysis method followed by a sucrose gradient centrifugation and western blot analysis. We found that Axl moved into the lipid raft-enriched fractions after 5 and 10 min of gas6 treatment as shown by its colocalization with caveolin-1. These results were confirmed by confocal microscopy. Interestingly, after gas6 treatment, c-Src, which is known to act as a transient docking platform for signaling molecules in lipid rafts, follow the same localization pattern to lipid raft-enriched fractions. The requirement of caveolae was then evaluated by transfecting HUVECs with caveolin-1 siRNA. Caveolin-1 knock down abolished completely gas6-induced phosphorylation of c-Src, Akt and ERK1/2 thus highlighting the role of caveolae in gas6-Axl signaling. Taken together these results demonstrate that activation of gas6-dependent signaling pathways involves Axl trafficking to lipid rafts/caveolae. These novel findings identify lipid rafts/caveolae as dynamic scaffolding systems for gas6/Axl interactions in endothelial cells.
Pro-Atherogenic Arsenic Exposure Alters Macrophage Polarization

Lemaire, M., Lemarié, C.A., Molina, M.F. and Mann, K.K.

Background: Environmental arsenic exposure is linked epidemiologically to increased atherosclerosis. Moreover, we showed that arsenic exposure altered plaque composition. However, the mechanisms by which arsenic enhances atherosclerosis are still unknown. Monocytes and macrophages are key players in atherosclerosis. Different macrophage phenotypes (M1, M2 or Mox) with different biological functions are present within atherosclerotic plaques. M1 are classical macrophages with inflammatory characteristics, M2 are reparative macrophages and Mox respond to oxidative stress in an nrf2-dependant manner (nuclear erythroid related factor-2). Objective: Arsenic activates nrf2, thus we hypothesize that it increases atherosclerosis by skewing macrophage polarization toward a Mox phenotype through nrf2 activation. Methods: We investigated the effects of arsenic on murine bone marrow derived macrophages by first culturing these cells in M-CSF into resting macrophages (M0) and then polarizing these into M1 with IFNγ or into M2 with IL-4. Macrophages or polarized macrophages were then exposed to arsenic (1.33 uM) and gene expression, phagocytosis and cholesterol transport were evaluated. Results: Arsenic-exposed naïve macrophages (M0) showed increased mRNA marker of Mox, including heme oxygenase-1. Furthermore, arsenic increased Mox markers in M1 and M2 macrophages, while decreasing markers of M1 and M2 (iNOS and FIZZ1, respectively), regardless of their primary polarization. Although these data suggest that arsenic skews macrophage differentiation toward Mox, we did not observe the characteristic decrease in phagocytosis following arsenic exposure. In addition, arsenic-exposed macrophages showed decreased pro-atherogenic cholesterol efflux. To better characterize the arsenic-enhanced plaque, we exposed ApoE−/− mice, a well-describe model of atherosclerosis, to arsenic (200 ppb) for 13 weeks and characterized the macrophage composition within the plaque. Conclusions: Arsenic may polarize macrophage to a distinct and unique class of macrophage characterized by high phagocytosis and increased nrf2 target genes. Our observations may lead to a better understanding of the role of macrophages in arsenic-induced atherosclerosis.
Adoptive transfer of T lymphocytes prevents angiotensin II-induced vascular stiffness in rag1 knockout mice

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Both innate and adoptive immune systems are involved in the pathogenesis of hypertension and vascular damage. T lymphocytes participate in the low-grade inflammatory response that contributes to vascular injury in cardiovascular disease, including hypertension. Angiotensin II-induced hypertension and endothelial dysfunction are blunted in rag1 knockout (rag1-/-) mice, which are deficient in T and B lymphocytes, and restored with adoptive transfer of T but not B lymphocytes. We hypothesized that adoptive transfer of T lymphocytes from C57Bl/6 mice (WT) will exacerbate angiotensin II-induced vascular damage in rag1-/- mice. Eleven-week old male rag1-/- mice were injected i.v. with PBS or 10 x 106 pan T lymphocytes, and, 2 weeks later, implanted with a dummy pump (control) or infused with angiotensin II (490 ng/kg/min, s.c.) for 14 days (n=5-8). Systolic (SBP) and diastolic blood pressure (SBP) were measured by telemetry. Endothelial function and vessel structure were assessed in second order mesenteric arteries by pressurized myography. NADPH oxidase activity was determined by lucigenin chemiluminescence. Successful adoptive transfer was confirmed at time of sacrifice by determining numbers of T cells in the spleen by flow cytometry. Angiotensin II increased SBP and DBP in rag-1-/- mice with adoptive transfer of WT T cells, whereas only SBP was increased in rag-1-/- mice injected with PBS. Angiotensin II impaired vasodilatory responses to acetylcholine in T cell-injected mice but not PBS injected mice. Adoptive transfer of WT T cells in rag-1-/- mice tended to restore angiotensin II-induced increase in NADPH oxidase activity in the kidney cortex and aorta. Angiotensin II treatment induced hypertrophic remodeling in PBS injected mice, but not in T cell-injected mice. Angiotensin II-induced vascular stiffness, indicated by a leftward shift of the small artery stress/strain relationship, was enhanced in the PBS injected mice but to a much lower extent in T cell-injected mice. These findings suggest that T lymphocytes have a protective role on angiotensin II-induced vascular stiffness.
Impact of Early Life BPA on Murine Cardiac Structure/Function

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Bisphenol A (BPA), an estrogenic endocrine disruptor, is used in the plastics industry and leaches out into the environment. BPA binds to estrogen receptors known to be present in fetal cardiomyocytes and appreciable amounts have been detected in the urine of individuals. Adult exposure is about 0.5?g/kg/day with exposure in children about 10-fold higher. We propose that BPA epigenetically reprogrammes early cardiac development to alter cardiac structure/function, DNA methylation and expression of proteins involved in calcium homeostasis. Pregnant C57bl/6n mice were exposed to Vehicle or oral BPA at childhood (5.0?g/kg/da), adult (0.5?g/kg/da or pharmacological (200?g/kg/da) doses starting on gestational day 11. Cardiac function was measured by tail cuff blood pressure, echocardiography and electrocardiography. Physical parameters were measured monthly until 4M. Organ weights were collected at euthanasia. Immunoblots of ventricle homogenates measured specific protein expression. We report the impact of BPA on adult male progeny. Prostate weight was 1.5-fold increased in BPA5.0 mice. Kidney and abdominal fat was 2-fold increased in BPA5.0 mice. BPA200 had reduced blood pressure suggesting some hypotension. A longer PR interval was observed in BPA5 males, suggesting a reduction in atria function. Echocardiography showed an increase in relative wall thickness in all BPA treated males suggesting cardiac hypertrophy. Fractional shortening, Vcf, pulmonary and ascending aorta VTI and cardiac output were similar suggesting no impact on ventricle function. SERCA2a and CASQ2 were increased >2-fold with no change in PLB or phosphorylated PLB at all BPA doses. This suggested an increased ability to restore calcium, as well as, store calcium in the SR. PLB, LTCC, NCX1 expression was unaffected. DNMT3a was 2-fold increased at all BPA doses and DNMT3b was reduced suggesting altered DNA methylation activity. Differences observed in cardiac function enable us to conclude that early life exposure to BPA, at BPA levels similar to those of childhood exposure, alters cardiac structure/function as well as cardiac gene expression in adult progeny. Such alterations are likely via changes in DNA methylation.
Transglutaminase 2 is a regulator of angiotensin ii-induced erk1/2 activation in vascular smooth muscle cells

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In essential hypertension, angiotensin (Ang) II induces arterial remodeling that is dependent on the activation of ERK1/2 pathway in vascular smooth muscle cells (VSMC). Arterial remodeling is also dependent on transglutaminase 2 (TG2), a protein with an emerging role in vascular pathophysiology. Beside its effect on extracellular matrix, TG2 is a multifunctional protein with GTPase activity and as such, could potentially be a signaling molecule. We hypothesized that TG2 mediates Ang II-induced ERK1/2 activation in VSMC during arterial remodeling. Methods and Results: To determine the role of TG2 in Ang II type 1 receptor (AT1R) signaling, His6 tagged-TG2 was overexpressed in the human embryonic kidney (HEK) 293 cell line stably overexpressing HA tagged-AT1R. Protein and phosphorylation levels were determined by Western blot. When stimulated for 2-60 min, TG2 overexpression potentiated 100 nM Ang II-induced ERK1/2 phosphorylation compared to control cells overexpressing green fluorescent protein (GFP, P<0.05, n=4). Maximal potentiation was observed after 2 min of stimulation at which time TG2 transfected cells showed an increase of 64±31% compared to GFP transfected cells. The role of TG2 in Ang II-induced phosphorylation of ERK1/2 was studied by TG2 siRNA knockdown in mouse vascular smooth muscle cell line (MOVAS). TG2 siRNA decreased TG2 expression 83±5% compared to cells treated with control siRNA (n=4). Transfection of TG2 siRNA did not significantly change cell viability when compared to ctrl siRNA (n=3). Ten min stimulation with 1 and 100 nM Ang II increased ERK1/2 phosphorylation 38±18% and 60±29%, respectively, and this was prevented by TG2 knockdown (P<0.01). Using western blots, HA-AT1R dimer/monomer ratio was increased by co-expression of His6-TG2 compared to HA-AT1R expressed alone. Conclusion: These results suggest that TG2 mediates ERK1/2 activation by Ang II through a mechanism involving TG2-induced AT1R dimerization. Overall, our results demonstrate that TG2 is involved in the activation of ERK1/2 in response to Ang II and could participate in arterial remodeling induced by Ang II.
Mapping of the region of chromosome 2 linked to vascular inflammation using congenic rats

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Immune cells have been implicated in hypertension and vascular inflammation. We demonstrated that chromosome 2 modulates immune responses in genetic hypertension via T regulatory lymphocytes (Treg). Introgression of chromosome 2 from normotensive Brown Norway rats (BN) into hypertensive Dahl salt sensitive (SS) background (consomic SB2) reduced vascular inflammation and restored Treg function. We hypothesized that the BN chromosome 2 contains genes that reduce vascular inflammation, which could be mapped using congenic rats containing portions of BN chromosome 2 on the SS background. Methods: Twelve-to-13 week old male BN, SS, SB2, congenic (SB)-A, SB-B and SB-E rats fed normal salt diet were studied. Systolic blood pressure (SBP) was measured by telemetry. Splenic Treg (CD4+CD25+) and effector (CD4+CD25-) T lymphocytes were isolated and characterized by FACS and cultured with CD3 activation. The TNF-a, IFN-g, IL-10, IL-17 and IL-6 in culture media was measured by microbead multiplex immunoassays. Aortic collagen content was determined by Sirius red staining. Results: SS, SB2 and SB-E exhibited 20 mmHg higher SBP compared to BN, SB-A, and SB-B (P
Endothelial Gas6 Contributes to Thrombogenesis and Promotes Tissue Factor Upregulation During Vessel Injury

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Gas6 is a vitamin K-dependent, secreted protein that amplifies platelet aggregation and secretion in response to platelet agonists. Gas6-/- mice are protected from experimentally induced lethal venous and arterial thromboembolism. This protection has been attributed to defective aggregation in platelets from gas6-/- mice. However, this platelet phenotype was only observed when platelets were challenged by only one agonist, ADP, and only at a concentrations of 5.0µM. This subtle platelet abnormality resulting in a rather dramatic clinical phenotype raise the possibility that gas6 from a source other than platelets contributes to thrombus formation. We hypothesize that gas6 derived from the endothelium plays a role in venous thrombus formation. Gas6-/- mice are protected against venous thrombosis induced by 0.37 M FeCl3 in the inferior vena cava (IVC). Bone marrow transplantation experiments generating mice with selective ablations of gas6 from either the hematopoietic or non-hematopoietic compartments demonstrate an approximately equal contribution by gas6 from both compartments to thrombus formation. Platelet depletion in wild type or gas6-/- mice followed by reconstitution with platelets from either WT or gas6-/- mice confirm that gas6 from compartments other than the platelet contribute to thrombus development. Furthermore, gas6-/- mice are hyporesponsive to FeCl3 mediated tissue factor induction in venous endothelium, as observed by immunofluorescence staining and later validated by a functional assay. In addition, in vitro, gas6-/- endothelial cells are hyporesponsive to thrombin mediated tissue factor mRNA induction. Taken together, these results suggest that non-hematopoietic gas6, possibly from the endothelium, contributes to thrombus formation in vivo and can be explained by the ability of gas6 to promote endothelial tissue factor induction. These findings support the notion that endothelial gas6 may play a pathophysiologic role in venous thromboembolism.
Recombinant Transferrin to which Iron is Bound Irreversibly Inhibits Iron Uptake by Erythroid Cells: Further Evidence for the “Kiss-and-Run” Hypothesis

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Delivery of iron (Fe) to most cells occurs following the binding of diferric transferrin (Tf) to its cognate receptors on the cell membrane. The Tf-receptor complexes are then internalized via endocytosis, and iron is released from Tf by a process involving endosomal acidification and reduction by Steap3. Iron is then transported across the endosomal membrane by the divalent metal transporter, DMT1. Unfortunately, the post-endosomal path of iron within cells remains elusive or is, at best, controversial. We have hypothesized that, in erythroid cells, mitochondrion-endosome interaction is involved in Fe translocation to its final destination and have collected experimental support for this proposition. We have previously shown, using 3D live confocal imaging, that the iron delivery pathway in reticulocytes involves a transient interaction of endosomes with mitochondria. Moreover, exploiting a novel method denoted “flow subcytometry”, we identified a double-labeled population representing endosomes (labeled by Alexa Green Transferrin [AGTf]) interacting with mitochondria (labeled by MitoTracker Deep Red [MTDR]) in reticulocyte lysates. The dynamic nature of this interaction was shown by chase experiments in which a time-dependent decrease of the double-labeled population was observed when reticulocytes were washed and re-incubated with unlabeled Fe2-Tf. To further prove our hypothesis, cell lysates from reticulocytes were analyzed by 2D confocal microscopy. Results from these experiments confirmed that fluorescently-labeled endosomes indeed come in physical contact with mitochondria. In addition, we have used different constructs of fluorescently labeled, recombinant human Tf, which either remain permanently bound to iron (recombinant diferric-transferrin; pb-FeTf) or cannot bind to iron (recombinant apotransferrin; rapoTf), in flow subcytometry studies. As expected, these studies showed that reticulocytes incubated with MTDR and rapoTf failed to produce a double-labeled population in uptake experiments. Interestingly, when reticulocyte lysates were incubated with MTDR and pb-FeTf, compared to controls using wild type human Tf, the size of the double-labeled population was decreased. This suggests that failure of iron release from pb-FeTf may interfere with the process of endocytosis or endosomal trafficking.
C/EBP alpha mediates shear stress-dependent downregulation of the angiotensin type 1 receptor

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The renin-angiotensin system, through the actions of angiotensin II, has significant proinflammatory actions on the vessel wall that can contribute to the progression of atherosclerosis. Hemodynamic forces are major determinants of atherosclerotic plaque localization. Plaques tend to form at arterial bifurcations, branch points, and curvatures where blood flow is low and oscillatory, whereas blood vessels exposed to high laminar shear stress remain comparatively devoid of plaques. Immunostaining of C57BL/6 mouse aortic arches showed a distinctive endothelial angiotensin type 1 receptor (AT1R) staining at the athero-prone inner curvature of the aortic arch but not in the adjoining outer curvature. In HUVECs exposed to laminar shear stress, we found that the AT1R was downregulated within 3 hours. The objective of the present study was to determine the mechanisms underlying shear stress-dependent downregulation of AT1R. We designed luciferase plasmids driven by different constructs of the AT1R promoter, spanning -1934 to +101 from the transcription start site, and transfected them into mouse primary endothelial cells. Using luciferase reporter assay, we found that shear stress reduced expression of the reporter construct by 42% (static = 3.00±0.51%, shear = 1.73±0.30%, P
IRP1-/- Mice Exhibit Defects in Glucose Metabolism and Erythropoiesis

Wilkinson, N. and Pantopoulos, K.

Iron Regulatory Proteins, IRP1 and IRP2, are post-transcriptional regulators of cellular iron metabolism that control the translation or stability of several Iron Responsive Element (IRE)-containing mRNAs. While IRP2-/- mice misregulate iron homeostasis in tissues and develop microcytosis, IRP1-/- counterparts have not been reported to manifest any overt phenotypic abnormalities. Recent in vitro experiments suggested that IRP1 preferentially regulates the expression of Hypoxia Inducible Factor 2 alpha (HIF2alpha or EPAS1) mRNA via its translation-type IRE (Zimmer et al, 2008). We therefore hypothesized that the disruption of IRP1 may interfere with HIF2a-dependent biological responses. To address this, we utilized IRP1-/- mice and focused on their glucose metabolism and erythropoiesis, which are controlled by known HIF transcriptional targets, the glucose transporter Glut1 and the hormone erythropoietin (Epo), respectively. IRP1-/- mice, IRP1+/- heterozygotes and wild type IRP1+/+ isogenic littermates (in C57B6 background) were first subjected to an oral glucose tolerance test. Compared to wild type controls and IRP1+/- heterozygotes, IRP1-/- mice presented significantly lower fasting glucose levels in the serum, as well as serum glucose levels at various time-points following gavage with a glucose bolus (Fig. 1). No differences were observed in serum insulin concentration among the three genotypes, indicating that the hypoglycemia seen in IRP1-/- mice was not a result of insulin upregulation. Analysis by qPCR showed a ∼2-fold increase of Glut1 mRNA in the kidney (p
Comparison of sexual activity and sexual dysfunction rates between women with scleroderma and women from a female population sample

Levis, B., Burri, A., Hudson, M., Baron, M. and Thombs, B.D.

Systemic sclerosis (SSc), or scleroderma, is a chronic, multi-system, connective tissue disorder characterized by thickening and fibrosis of the skin and internal organ involvement. Approximately 80% of patients are women, with highest onset rates between ages 30-60. Common problems include pain, fatigue, pruritus, body image distress, depressive symptoms, and general disability. Reduced sexual activity and impaired sexual functioning are also common among women with SSc and are associated with age and marital status, in addition to disease symptoms (Levis et al., Arthritis Care & Research, 2012). To date, however, there have not been any population data available to compare rates of sexual activity/impairment among women with SSc and women from a non-medical population sample. Thus, the objectives of this study were to 1) determine the rates of sexual activity and sexual impairment, stratified by age and marital status, for a SSc sample and a female population sample, and 2) identify the odds of sexual activity and sexual impairment for women with SSc compared to the population sample, controlling for age and marital status.

Methods: We compared female SSc patients from 12 Canadian Scleroderma Research Group Registry centers to a UK female population sample. SSc patients underwent medical examinations and clinical histories and were asked whether they had engaged in sexual activities with their partner in the past 4 weeks. Sexually active patients completed a 9-item version of the Female Sexual Function Index (FSFI). Women from the UK completed the FSFI as part of a larger study on the genetic/environmental influences underlying female sexual dysfunction. Multivariate logistic regression analyses were used to assess the independent contributions of sample group, age and marital status to sexual activity and impairment status. Results: Overall, among 730 women with SSc, 296 (41%) were sexually active, 181 (25%) of whom were sexually impaired. Among 1498 women in the population sample, 956 (64%) were sexually active, 420 (28%) of whom were impaired. For each age group/marital status combination, women with SSc were less likely to be sexually active and more likely to be sexually impaired. Controlling for age and marital status, women with SSc were significantly less likely to be sexually active (OR=0.34, 95%CI=0.28-0.42) and significantly more likely to be sexually impaired (OR=1.88, 95%CI=1.42-2.49). Conclusions: This study highlights that sexual functioning is a problem for many women living with scleroderma. Research should be done to develop interventions to improve sexual functioning among women with this disease.
Sleep Disturbances in Systemic Sclerosis: Evidence for the Role of Pain and Gastrointestinal Symptoms

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Systemic Sclerosis (SSc) is an autoimmune connective tissue disorder characterized by thickening and fibrosis of the skin and internal organs. There is significant mortality and morbidity in SSc and no cure. Consequently, improving symptom management is critical for reducing disability and improving patient quality of life. Problems that contribute to a reduced quality of life in SSc include fatigue, pain, depression, sexual dysfunction, body image distress, and itching. Sleep disturbances also appear to be common in SSc however there has been very little research in this area. The objective of this study was to investigate socio-demographic and medical factors associated with sleep disturbance in a large, pan-Canadian SSc sample using an ‘a priori’ model and a validated, self-report sleep disturbance measure. Methods: Patients who completed annual visits as part of the Canadian Scleroderma Research Group’s (CSRG) Registry were included in the study. Registry patients must have a diagnosis of SSc made by a Registry rheumatologist, be >18 years of age, and be fluent in English or French. The 8-item PROMIS sleep disturbance measure assesses perceptions on sleep over the past 7 days. Scores range from 8 to 40 with higher scores indicating more disruption. Analyses of potential correlates of sleep disturbance included self-reported socio-demographic variables, as well as medical variables obtained through clinical histories and medical examinations. Multivariable associations of general population (step 1) and medical (step 2) variables with PROMIS sleep disturbance scores were assessed using hierarchical multiple linear regression. Results: The mean (SD) sleep disturbance score was 22.8 (23.0). Only the number of gastrointestinal (GI) symptoms (standardized β=0.20, p
Experiencing Brachytherapy for Rectal Cancer: The Patient’s Perspective

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Rectal cancer Brachytherapy involves: placing a radioactive material inside the rectum as closest to the tumor, using a higher total dose of radiation to treat a smaller area and in a shorter time than is possible with external radiation. The primary aim of this study was to assess patients’ experience during rectal brachytherapy, more specifically, exploring their thoughts, perceptions, emotions and physical sensations while receiving their treatment. The second aim was to report on how rectal cancer patients cope with this complex procedure. Methods: Twenty-five patients (Mage = 60.50, SD = 11.01) diagnosed with rectal cancer who received brachytherapy treatment were recruited from the radiation oncology department of the Jewish General Hospital. Semi-structured qualitative interviews were conducted with 25 patients following completion of treatment. Interviews were transcribed and analyzed using thematic-content analysis. Results: Thematic-content analyses of post treatment interview transcripts identified the emergence of 7 core themes: 1) pain, 2) temporality, 3) patient needs, 4) relation with the staff, 5) technical procedure, 6) self thoughts and 7) embodied state. Each theme was further subcategorized to identify both collective and idiosyncratic dimensions. Selected quotes from each transcription will be presented to illustrate the aforementioned themes. Conclusion: Results indicate that for the majority of patients, pain is associated with the insertion of the applicator. Notably, there was great variation of pain across individuals in relation to gender and cultural groups. The experience described often uses metaphors and analogies. Additionally, emotions were expressed as being embodied within pain, and for some there is a fusion between specific emotions (ex: anxiety, stress) and pain. Perception of time is a moderator for how much discomfort ones can tolerate. Research Implications: The cultural variation in idioms used to express pain needs further exploration. Additionally, further research is needed about ways of addressing needs and word choices from the treatment team that triggered comfort and avoided further. Using the images and metaphors provided by patients can help researchers develop relaxation and hypnotic interventions to be used during treatment. Clinical Implications: The medical team needs to listen to patients words as subjective indicators of the modulation of their individual pain and discomfort. The needs of patients (e.g. clock, discomfort at the hips from positioning) suggests that improvements can be made to cancer care. Additionally, results be used to develop the unique wording of psychosocial interventions (i.e hypnotic inductions, self regulation techniques) used during treatment.
The PHQ-9 Versus the PHQ-8 – Is Item 9 Useful for Assessing Suicide Risk in Coronary Artery Disease Patients? Data from the Heart and Soul Study

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Item 9 of the Patient Health Questionnaire-9 (PHQ-9), which inquires about both passive thoughts of death and active ideas of self-harm, has been used to assess suicide risk. The objectives of this study were (1) to determine the proportion of patients who responded “yes” to Item 9 who endorsed active suicidal ideation in response to more direct questions from a structured clinical interview and (2) to compare the sensitivity and specificity for detecting cases of depression of the PHQ-9 and the PHQ-8, which does not include Item 9, as well as the correlation between the PHQ-8 and PHQ-9. Methods: Coronary artery disease (CAD) outpatients were administered the PHQ-9 and the Computerized Diagnostic Interview Schedule (C-DIS). Item 9 responses were compared to suicidal ideation and intent in the last year based on the C-DIS. Scores on the PHQ-8 were obtained by eliminating Item 9 from the PHQ-9. Test characteristics of the PHQ-9 and PHQ-8 were compared. Results: Of 1,022 patients, 110 (10.8%) endorsed Item 9. Of those, only 22 (19.8%) reported thoughts about committing suicide, and only 9 of those (8.1%) reported a suicide plan any time in the last year based on the C-DIS. Correlation between PHQ-9 and PHQ-8 scores was r=0.997. Sensitivity and specificity for the PHQ-9 (54%, 90%) and PHQ-8 (50%, 91%) to detect major depression were similar. Conclusion: Item 9 does not appear to be an accurate suicide screen. The PHQ-8 may be a better option than the PHQ-9 in CAD patients.
Deconstructing Human Papillomavirus (HPV) Knowledge: Objective and Perceived Knowledge in Males’ Intentions to Receive the HPV Vaccine

Stephenson, E., Krawczyk, A., Perez, S., Lau, E. and Rosberger, Z.

Recently approved and recommended for men in Canada, the human papillomavirus (HPV) vaccine protects males against HPV-related cancers (e.g., oral, penile, and anal) and genital warts. Further, male vaccination may prevent the spread of HPV, which reduces the risk of cervical cancer in women through herd immunity. To effectively tailor HPV education efforts to men, it is important to understand what men know about HPV and how this knowledge relates to their decision to receive the vaccine. This study examines how objective HPV knowledge, objective HPV vaccine knowledge, and perceived knowledge (level of confidence in one’s HPV knowledge) relate to males’ HPV vaccination intentions. METHODS: One hundred and twenty eight college men (M age = 20.8, SD = 2.04) completed a questionnaire survey assessing their demographics, sexual health, and objective and perceived knowledge of HPV and the HPV vaccine. They were also asked whether or not they intended to receive the HPV vaccine. RESULTS: Forty two percent of participants intended to receive the HPV vaccine. There were significant knowledge gaps, with low objective and perceived knowledge overall. Logistic regression analyses revealed that perceived knowledge and objective HPV vaccine knowledge were associated with vaccination intentions. Perceived knowledge was significantly associated with vaccination intentions even when accounting for objective HPV and HPV vaccine knowledge. DISCUSSION: Perceived knowledge may play an especially important role in motivating men to receive the HPV vaccine. Educational programs that aim to increase HPV vaccine acceptability should foster individual’s confidence in their HPV knowledge, beyond providing factual HPV-related information. It is important that men feel confident in their knowledge about HPV and the HPV vaccine, because it is not only what they know, but also what they think they know that is related to vaccination intentions.
Interventions for implementation of thromboprophylaxis in hospitalized medical and surgical patients at risk for venous thromboembolism: A Cochrane Review


Prophylactic therapies for venous thromboembolism (VTE) are effective and safe, yet, underutilized. There are many proposed strategies to increase the use of thromboprophylaxis. System-wide interventions, administered at the hospital or departmental level, may be more effective to improve the use of VTE prophylaxis than relying on individuals’ prescribing behaviors. Objectives: To determine the effectiveness of various interventions designed to increase the use of thromboprophylaxis in hospitalized patients at risk for VTE. Methods: MEDLINE, EMBASE, and SCOPUS databases and reference lists of included studies and published reviews were searched. Eligibility, data extraction, and risk of bias were assessed in duplicate. Primary outcomes included the proportion of patients who received prophylaxis (RP) and received appropriate prophylaxis (RAP). Meta-analyses of RCTs and non-randomized studies (NRS) were conducted separately and interventions were categorized into education, alerts, and multifaceted interventions. Random effects models were used to pool risk differences (RD) and heterogeneity was assessed using the I² statistic and subgroup analyses. Results: Of 1802 records included in the primary screen of titles and abstracts, 78 studies were deemed potentially relevant and their full-text articles were assessed for eligibility. Fifty-six studies (8 RCTs and 48 NRS) were included in the review. Among RCTs, there were sufficient data to pool one outcome (RP) for the intervention alert. Alerts, such as computerized reminders or stickers on patient’s charts, were associated with a 13% increase in the proportion of patients who received prophylaxis (95% CI: 6%-21%). Among NRS, there were sufficient data to pool both primary outcomes for each intervention type. Pooled RDs for RP ranged from 8% to 15%, and for RAP ranged from 12% to 19%. Education and alerts were associated with statistically significant increases in prescription of appropriate prophylaxis and multifaceted interventions were associated with statistically significant increases in prescription of any prophylaxis and appropriate prophylaxis. Multifaceted interventions had the largest pooled effects. I² results showed substantial statistical heterogeneity which was in part explained by patient types and type of hospital. Conclusions: We found significant increases in prescription of prophylaxis and appropriate prophylaxis associated with education, alerts, and multifaceted interventions. Multifaceted interventions that included an alert component appeared to be more effective (as suggested by greater pooled RD) than alerts alone or education alone. Our results suggest that any intervention can be effective, but multifaceted approaches appear to have the greatest effect.
The human leukocyte antigens (HLA) have roles in immune function and histocompatibility and are divided into HLA-I and HLA-II. Soluble forms of HLA-II (sHLA-II) have been detected in body fluids such as tears, saliva, and spinal fluid, while sHLA-I has been detected in highest concentrations in serum. HLA-G, a member of the non-classical subgroup within the class of HLA-I, has been implicated in the development/maintenance of autoimmune and inflammatory conditions. HLA-G has been detected in various organs during inflammatory processes that often have an autoimmune etiology. Increased levels of serum sHLA-G have been reported to correlate with disease activity/severity in rheumatoid arthritis patients. HLA-G expression in chronic inflammatory diseases has been associated with a better clinical course. In systemic sclerosis (SSc), HLA-G expression in skin correlated with a lower frequency of vascular and visceral disease involvement. Objectives: 1) Compare the levels of sHLA-G in saliva and serum among SSc patients and controls, 2) Assess a correlation of sHLA-G levels between serum and saliva and 3) Determine if the levels of sHLA-G in serum and saliva of SSc patients are associated with disease severity. Methods: Scleroderma patients were enrolled from the Canadian Scleroderma Research Group while controls were recruited from the rheumatology clinic of the Jewish General Hospital. Patients were asked to give 5 ml of saliva in a test tube, to complete a questionnaire, and to give a sample of blood. Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine levels of sHLA-G in both serum and saliva. Statistical analyses were performed using SAS version 9.2. Results: Forty-three SSc patients and 17 controls were included in the study. After adjusting for age and gender, the salivary sHLA-G levels were higher in SSc patients than controls (p = 0.02), but not in serum (p=0.59). A significant difference in salivary sHLA-G levels was observed between controls and SSc patients with non-severe disease (p = 0.04), but this was not the case for severe SSc (p = 0.17). The levels of salivary and serum sHLA-G were correlated (r = 0.45, p = 0.0004). Smoking and anti-inflammatory drugs were not confounders. Conclusions: These results indicate that there are differences in levels of sHLA-G between SSc patients and controls, and that the differences observed vary in serum and saliva. A bigger sample size will be needed to complete this analysis.
Immigrants have higher mortality from chronic viral hepatitis and hepatocellular carcinoma as compared to those born in Canada. Approximately 20% of this burden is likely due to hepatitis C (HCV). Despite this disparity there are no screening programs to detect chronic HCV for immigrants after arrival in Canada. This is due to a lack of population based data describing the burden of chronic hepatitis C in immigrants. To fill this gap, we created a cohort of all cases of hepatitis C reported from 1998-2007 in Quebec using the MADO (Quebec Reportable Disease) database linked to two other Quebec administrative databases; the MICC (Quebec Landed Immigrant) and the RAMQ (Quebec provincial health insurance and physician billing) databases. Incidence rates were estimated using the reported number of cases in immigrants and non-immigrants during the study period. Quebec census data from 1996, 2001, and 2006 (stratified by immigrant and non-immigrant status) was used for the denominator. Rates, rate ratios, and 95% CI were calculated using the Poisson distribution. A total of 20,459 cases of chronic hepatitis C (1,980 immigrants and 18,479 non-immigrants) were reported between 1998-2007. Immigrant cases were older (mean age 47.1 vs 43.1 years, p-value < 0.0001) and were less likely to be male (53.1% vs 68.2%, p-value)
Mutation is the ultimate source of genetic variation, which could be deleterious to the genome or provide the fuel for phenotypic evolution. The understanding of the nature of human mutation accumulation is essential to many aspects of medical genetics and human evolution. Germline mutation rates have been widely studied by utilizing the data at loci causing Mendelian disease, comparing putatively neutral evolving nucleotide sequences between humans and chimpanzees, and comparing the genome sequences of relatives. The estimate of human mutation rate per nucleotide site, per generation is consistent from these different approaches, which has been suggested to be $\sim 10^{-8}$. However the somatic rate, which is distinct from the germline mutation rate, has not been thoroughly investigated in humans, but is central to the etiology of somatic diseases, such as cancer. In this study we genome-wide genotyped 92 pairs of monozygotic twins and directly measured the somatic mutation rate by single base substitution in 506,786 high quality genotype sites. The confirmation of candidate mutations identified from genome-wide genotyping discrepancies was carried out by Sanger sequencing in 33 pairs of monozygotic twins for whom DNA is available. Two somatic mutations were verified from two distinct pairs of twins. It is likely that these two mutations occurred early in embryonic development since they did not occur in loci likely to confer survival advantage and Sanger sequence is only able to identify mutations that are present in the majority of a cell population. The estimated somatic mutation rate derived from these observations is $1.20 \times 10^{-7}$, which is similar to the mutation rate quantified using cell lines ($10.6 \times 10^{-7}$). These data allow us to estimate that each individual carries, on the average, 359 post-zygotic mutations that happened early enough or conferred enough of an advantage to be present in the majority of blood cells. These findings provide direct evidence that somatic mutations do occur and can lead to differences in genomes between otherwise identical twins, suggesting that mosaicism due to such mutations is likely present among the trillions of mitosis that occur over the human lifespan. This study was funded in part by Canadian Institute of Health Research.
Studies have looked at several factors that may affect the risk of developing prostate cancer. One of these factors includes type 2 diabetes mellitus (T2D). T2D is known to be associated with an increased risk of developing several cancers, including colorectal and endometrial cancer (1). However, the risk of developing prostate cancer was found to be 30% less in patients with T2D (2). This finding has not been consistent, as other studies showed no relation between the incidence of prostate cancer and T2D (3,4). Therefore, it is not clear whether T2D decreases the risk of developing prostate cancer. Using novel genetic epidemiology methods we sought to understand whether the alleles increasing risk of T2D, and related traits, also increased the risk of prostate cancer. Methods: Using evidence from the literature, we identified alleles that are associated with increased risk of T2D and related traits (fasting glucose levels, glycemic levels 2 hours post glucose load, glycated hemoglobin A1c levels (HbA1c), and HOMA-B levels) at a genome-wide significant level. We then used a multi-SNP genotypic risk score to test the association of risk alleles in each category to determine the effect the glycemic trait has on the risk of developing prostate cancer using data from the CRUK cohort genome-wide association study (GWAS) for prostate cancer (5). Results: The multi-SNP genotypic risk scores calculated for risk alleles for T2D, fasting glucose, and HbA1c levels were associated with a decreased risk of developing prostate cancer but did not reach statistical significance. The risk score calculations for HOMA-B showed a positive association on the risk of developing prostate cancer but this result was also not statistically significant. Conclusions: T2D, fasting glucose and Hba1c may have an inverse relationship with prostate cancer risk, whereas HOMA-B levels may have a direct relationship with the risk of developing prostate cancer. These results suggest that traits associated with hyperglycemia may decrease prostate cancer risk. We are currently awaiting data from the PRACTICAL Consortium (Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome), which will have a larger sample size to see if the effects observed in this study will reach statistical significance. References: 1. Johnson JA et al. (2011) Time-varying incidence of cancer after the onset of type 2 diabetes: evidence of potential detection bias. Diabetologia. 54:2263-2271. 2. Adami HO, et al. (1991) Cancer risk in patients with diabetes mellitus. Cancer Causes Control 2: 307-314. 3. Will JC et al. (1999) Is diabetes mellitus associated with prostate cancer incidence and survival? Epidemiology 10: 313-318. 4. Tavani A et al. (2002) Diabetes and the risk of prostate cancer. Eur J Cancer Prev 11:125-128. 5. Eeles et al. (2009) Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. Nature Genetics 41(10):1116-1121
Performance of Genotype Imputation for Rare Variants

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Common variants explain little of the variance of most common disease; prompting large-scale sequencing studies to understand the contribution of rare variants to common disease. Since large sample sizes will be required for most sequencing studies, imputation of rare variants from genome-wide genotypic arrays offers a cost-efficient strategy to achieve necessary sample sizes, however the accuracy of imputation of rare genetic variants is not known. Objective: To estimate the accuracy of imputation of rare variants using different publicly available reference panels of varying sample size and genome-wide genotype arrays of varying SNP density. Methods: Using 153 participants, each of whom was genotyped on 3 different genotype arrays including 317k, 610k and 1 million SNPs, we imputed to four different reference panels: HapMap2, 1000 Genomes pilot (1KGpilot), 1000 Genomes interim August 2010 release (1KGinterim), and 1000 Genomes phase1 November 2010 release (1KGphase1) using IMPUTE version 2. The differences between these three releases of 1000 Genome data are the sample size, number of variants and their frequency spectrum. Results: More than 94% of total SNPs yield an acceptable imputation quality (info>0.4) in HapMap based imputation. For the 1000 Genome based imputation, the proportion of well-imputed SNPs in 610k imputations was 94%, 84%, 72% for SNPs with MAF from 1% to 5% for 1KGphase1, 1KGinterim and 1KGpilot respectively. This proportion of well-imputed SNPs dropped to 85%, 60% and 45% for SNPs with MAF from 0.5% to 1%; and 62%, 33% and 30% for SNPs with MAF from 0.3% to 0.5% for the same reference panels. For SNPs with MAF = 0.3%, only 1% of the variants were well imputed in 1KGphase1 and 1KGinterim imputations, and none are well imputed in 1KGpilot imputation. The proportion of the high quality SNPs increased as the density of the genotypes increased across genotyping arrays, but the differences were small compared to effect of reference size. Conclusions: The imputation accuracy of variants with a MAF from frequency of singletons to 0.05 improves with an increasing size of reference panel, and increasing density of genome-wide genotyping arrays. Despite large reference panel size and dense genotyping density, very rare variants remain difficult to impute. These findings have important implications in the design and replication of large-scale sequencing studies.
DNA methylation, the addition of methyl groups at CpG sites in the genome, is one of the most studied epigenetic marks. Alterations in methylation levels have been shown to cause several human diseases through transcriptional aberrations. For instance, hypermethylation of CpG islands located in the promoter regions of tumor suppressor genes has been firmly established as one of the most common mechanisms for gene regulation in cancer (Esteller, 2007; Herman et al, 2003). A popular robust methylation profiling platform via bisulfite conversion is the Illumina Infinium Methylation Assay. The recently released Infinium HumanMethylation450 BeadChip Kit allows the interrogation of more than 450,000 methylation sites per sample. Because genome-wide DNA methylation levels can vary substantially from one person to another, and because a large proportion of CpG sites will have at least some methylation, the normalization procedures often used for gene expression studies are not appropriate and need to be modified (Siegmund, 2011). In particular, the presence of two chemistry technologies in the HumanMethylation450 BeadChip has implications for the within-array normalization as well. Results: We propose a normalization procedure based on several negative control probes that takes into account the two kinds of probes, as well as correcting for background effects, dye bias and batch effects. We apply the model to methylation array results from stored lymphocytes of approximately 2000 individuals (1000 from cases of colorectal cancer, 1000 from control individuals) using two different metrics: the M-value, which is the log-ratio of the intensities of the methylated probe versus the unmethylated probe, and the Beta-value, which is the ratio of the methylated probe intensity and the overall intensity. We then perform a differential methylation analysis for cases and controls, while examining relationships with other covariates known to affect colorectal cancer risk. We compare the two metrics in order to decide which one is a better choice for analysis, or whether alternative metrics should be used. For each metric, we will compare the results obtained with usual normalization procedures and with the model that we propose. Acknowledgements: Special thanks to Mathieu Lemire, Steve Gallinger, Brent Zanke, Tom Hudson and the ARCTIC project (Assessment of Risk in Colorectal Tumours in Canada).
Molecular profiling of acquired resistance to paclitaxel in triple negative breast cancer cell lines

Ahmadzadeh, E., Przybytkowski, E., Aguilar-Mahecha, A. and Basik, M.

Triple-negative breast cancers (TNBC), accounting for 10-20% of breast cancers, show a very aggressive clinical behavior with distinctive metastatic patterns and poor prognosis. Patients with TNBC do not benefit from hormonal therapies or treatments targeted against HER2 because their tumors do not express estrogen and progesterone receptors and have very low levels of HER2. TNBCs may have a good initial response to chemotherapy, particularly taxane-based therapy. However, the propensity to recur after initial response to chemotherapy and to acquire resistance to subsequent therapies is the major obstacle in successful treatment. To better understand the molecular mechanism of resistance to Paclitaxel (PTX) in TNBCs we developed an in vitro model of PTX-resistance. A series of 7 TNBC cell lines were selected and the resistant counterparts were generated. Our goal is to find molecular factors associated with PTX resistance, which can be further explored as biomarkers or drug targets in resistant TNBC. The parental cell lines show great variability in the response to PTX; the IC50 varies from picomolar to micromolar values. The newly generated PTX-resistant cell lines are 100 to 10000 fold more resistant to PTX as compared to its parental cells. Characterization of resistant cells show phenotypical changes and growth rate reduction as well as changes in cell cycle distribution and ploidy. Also, the expression of β-tubulin isotypes, known to be related to taxane-resistance in cancer cells, is significantly altered in resistant cells. In addition, comparison of chromosomal alterations in drug-resistant and parental cell lines by comparative genomic hybridization showed amplifications or deletions of chromosomal segments, which may involve novel genes associated with drug resistance. We will further continue to profile the parental and resistant cells in a multi-dimensional approach (exome-capture sequencing, array CGH, gene expression, microRNA and phosphoproteins) in order to identify molecular changes appearing in the resistant cells.
Identification of a ShcA-driven cytokine response that facilitates immunosuppression during mammary tumorigenesis

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Early breast cancer lesions stimulate the recruitment of immune cell types, called cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, which recognize and destroy cancer cells through a process termed immune surveillance. Eventually, cancer cells acquire the ability to escape these immune-protective effects and progress to clinically detectable tumors through a process termed immunosuppression. This is achieved either through the selection of tumor cells that no longer present tumor antigens to CTLs or that induce the production of immunosuppressive cytokines. Using ShcA-deficient transgenic mouse models, we have previously shown that this adaptor protein engages signaling pathways in breast cancer cells that are required for the establishment of an early immunosuppressive state1. However, the molecular mechanism by which the ShcA pathway limits the recruitment of CTL to favour mammary tumorigenesis is unclear. We demonstrate that decreased expression of ShcA within breast cancer cells, using shRNA approaches, reduces tumor outgrowth and significantly increases CD8+ T cell infiltration in syngeneic mouse models of the disease. We further demonstrate that these ShcA-deficient breast cancer cell lines produce significantly elevated levels of two IFN-γ regulated chemokines, CXCL9 and CXCL10, relative to their wild-type counterparts. Importantly, these chemokines are known to selectively recruit activated T lymphocytes and have previously been shown to confer anti-tumorigenic properties. These data suggest that ShcA signalling in breast cancer cells establishes a pro-tumorigenic microenvironment by limiting the production of tumor suppressive chemokines to ensure that tumor cells establish immunosuppression early during disease progression.
Targeting Trastuzumab resistance in HER-2+ breast cancers with the reversible pan-erbb inhibitor AZD8931

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Approximately 15-20% of all invasive breast carcinomas overexpress the HER-2 receptor, a membrane surface-bound receptor tyrosine kinase upstream of critical proliferation and cell survival pathways. Trastuzumab, a humanized monoclonal antibody to the extracellular domain of HER-2, has proven to be a beneficial treatment for patients diagnosed as HER-2+, but does have its limitations. A majority of advanced HER-2 positive breast cancer patients will develop resistance to the therapy within the first year while many others do not respond to the drug when used alone. The purpose of this study was to determine whether AZD8931, a reversible pan-ERBB inhibitor that equipotently inhibits EGFR, HER-2, and HER-3-mediated signaling, could reverse this resistance in established trastuzumab-resistant BT474 and SKBR3 cell lines. It was also to compare the activity of AZD8931 with Lapatinib, a reversible inhibitor of EGFR and HER-2 that is currently used in the clinic for HER-2+ patients who progress on trastuzumab but possesses toxic side effects. To account for tumor heterogeneity in HER-2+ tumors, response to AZD8931 was examined in both ER-positive (BT474) and ER-negative (SKBR3) cell lines. Proliferation assays were used to assess the efficacy of AZD8931 and Lapatinib alone and in combination with trastuzumab. Results showed significantly diminished cell growth in all tested cell lines, including those resistant to trastuzumab, at clinically relevant concentrations of both AZD8931 and Lapatinib.
Linear ubiquitination of NEMO negatively regulates the interferon antiviral response through disruption of the MAVS-TRAF3 complex

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The RIG-I/mda5 sensors recognize RNA virus infection through their ability to bind intracellular viral RNA and trigger the host antiviral response. In the present study, we investigated the role of linear ubiquitination in controlling RIG-I signaling. The LUBAC complex, consisting of the E3 ligases HOIL-1L, HOIP, and the accessory protein SHARPIN, differentially regulated the RIG-I signaling pathway and downregulated virus-mediated interferon (IFN) induction by targeting NEMO for linear ubiquitination. Linear ubiquitinated NEMO associated with TRAF3 and disrupted the MAVS-TRAF3 complex leading to the inhibition of IFN activation, while stimulating NF-kB dependent signaling. Additionally, decreased VSV replication, concomitant with increased IFN production, was observed in MEFs deficient in SHARPIN. This study highlights a crucial role for linear ubiquitination of NEMO in the negative regulation of the IFN antiviral response.
The Human Telomerase ‘Insertion In Fingers Domain’ Is Implicated In The Mediation Of Telomerase-Specific Functions, Cancer And Premature Aging Diseases

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As a requirement for telomere maintenance, cellular immortalization and carcinogenesis, telomerase activation is a predominant tumor biomarker detected in over 85% of human cancers. Minimally composed of the catalytic subunit TERT, telomerase uses a short integral RNA to template telomere synthesis (1). However, TERT is also distinguished by a sizable insertion between the conserved motifs A and B’ of the “palm” and “fingers” subdomain, referred to as 'the insertion in fingers domain' (IFD). Telomerase-specific functions include the ability to repeatedly translocate and catalyze the reiterative addition of short telomeric DNA sequences onto the same DNA substrate using an intrinsic RNA template (repeat-addition processivity (RAP)) (1). In yeast, the IFD region has been implicated in enzyme processivity and telomere maintenance (2). We hypothesize that the distinctive IFD region modulates enzymatic functions unique to telomerase which contribute to telomere maintenance and human cellular immortalization. We generated a panel of 15 substitution mutations in the human TERT (hTERT) IFD region based on previous mutations made in yeast, on sequence conservation and on mutations associated with premature aging syndromes. In vitro-reconstituted mutant telomerase complexes were assessed for telomerase activity and processivity by the telomeric repeat amplification protocol and the direct primer extension assay, respectively (1). Our preliminary data shows that 5 mutants display greater than 50% decrease in activity and most displayed wild-type processivity, with the exception of two mutants, L805A showing dramatic defects in RAP and V763S, displaying 1.5 fold increase in enzyme processivity. Furthermore, using a primer binding assay, we found that L805A has ~2 fold increase and V763S has a slight decrease in binding to a telomeric substrate compared to wild-type hTERT. We speculate that the strong interaction of the L805A with the substrate may impair the translocation step while the slightly lower binding affinity of the V763S may potentially facilitate this rate-limiting step. The extent of telomere maintenance and cellular immortalization mediated by these mutant telomerases is currently being assessed. Deciphering the underlying role of the IFD in mediating telomerase-specific functions will provide a molecular framework that will serve as a foundation for the design of unique telomerase-based therapies. (1) Moriarty, T. J., et al. 2005. Mol. Biol. Cell. 16:3152-61. (2) Lue, N. F., Y. C. Lin, and I. S. Mian. 2003. Mol. Cell. Biol. 23:8440-49.
The epithelial-mesenchymal-like transition (EMT) is a process enabling epithelial cells to gain the motile characteristics of mesenchymal cells, in a manner resembling metastasis. TGF-beta, via well-defined transcriptional mechanisms, is considered a master regulator of EMT. However, the idea that TGF-beta can regulate the translational machinery to drive EMT remains largely unexplored. The eukaryotic translation initiation factor eIF4E is a protein known to be overexpressed in breast cancer, has been linked to increased invasiveness, and is a promising target for the treatment of breast cancer. Our hypothesis is that phosphorylation of eIF4E stimulated by TGF-beta is required for inducing EMT and metastasis in breast cancer. Our novel preliminary data show that TGF-beta can stimulate eIF4E phosphorylation as normal epithelial cells become mesenchymal. Moreover, silencing of eIF4E attenuates molecular and behavioral changes associated with EMT. In keeping with a role of phosphorylated eIF4E in driving the metastatic phenotype, we show that chemically and genetically inhibiting the eIF4E kinase MNK1, attenuates TGF-beta-stimulated EMT. Moreover, silencing of eIF4E in invasive breast cancer cells can suppress the mesenchymal phenotype. This research is directly relevant to breast cancer research because: (1) eIF4E is overexpressed in approximately 50% of breast cancers, (2) phosphorylated eIF4E is essential for tumor progression and invasion, (3) clinically, eIF4E has been proposed as a therapeutic target to treat human malignancy, and (4) TGF-beta signaling is required for EMT and breast tumor metastasis.
Stress-induced degradation of newly synthesized polypeptides is mediated by ribosome-associated RACK1/ JNK/ eEF1A2 complex

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Folding of newly synthesized polypeptides into functional proteins is a highly regulated process. Sizable amount of newly synthesized polypeptides (NSPs) are degraded during or shortly after completion of translation. This is thought to be as an essential part of the quality control mechanism, which ensures that newly synthesized proteins attain their native fold. Nonetheless, signaling pathways that govern degradation of NSPs in mammals remain elusive. We demonstrate that the stress-induced Jun N-terminal kinase (JNK) is recruited to polysomes by receptor for activated C kinase (RACK1), an integral component of the 40S ribosome and adaptor for protein kinases. Ribosome-associated JNK phosphorylates eukaryotic translation elongation factor 1A isoform 2 (eEF1A2) on serines 205 and 358, which promotes the association of eEF1A2 with NSPs, and accelerates their degradation by proteasomes. These findings establish undisclosed regulatory mechanisms, which govern degradation of NSPs in mammals.
Darinaparsin overwrites HMOX-1 mediated cytoprotection via DNA damage and cell cycle arrest related inactivation of BRG1 in AML


Arsenic trioxide (ATO) is a successful treatment option for Acute Promyelocytic Leukemia (APL), but remains mildly effective in other cancers. Moreover, APL cells can develop resistance to ATO. It has been shown that resistance to ATO and/or moderate sensitivity correlates with increased expression of cytoprotective enzymes. Darinaparsin (Dar) is a significantly more potent apoptosis-inducing arsenical than ATO in various malignant cell lines and in blasts coming from APL patients. Interestingly, ATO-resistant APL cells are sensitive to Dar. We hypothesized that the increased cytotoxicity of Dar may be due to a decreased cytoprotective response, and therefore studied the expression of the stress response enzymes after ATO and Dar treatment. We observed a significantly higher increase in expression of the nuclear factor (erythroid-derived 2)-like 2 phase-II (Nrf2)-dependant detoxifying enzymes with Dar than with ATO, with the notable exception of heme-oxygenase 1 (HMOX-1), which was not induced by Dar. Even though the Nrf2 signaling cascade was activated by Dar treatment, Nrf2 was not recruited to the HMOX-1 promoter’s antioxidant response elements (AREs). We also found that Dar treatment did not lead to the recruitment of Brahma-related gene 1 (BRG1) to HMOX-1 AREs. We observed that, whereas ATO induces a cell cycle arrest in G1, Dar induces a cell cycle arrest in G2/M. We showed that during G2/M, BRG1 is hyper-phosphorylated and excluded from the chromatin, which prevents HMOX-1 induction. In order to elucidate whether the lack of HMOX-1 expression or activity contributes to the increased efficacy of Dar, we examined the response to ATO and Dar upon co-treatment with the HMOX-1 inhibitor OB-24 and upon shRNA knock-down. We showed that pharmacological inhibition and expression knock-down of HMOX-1 increased the toxicity of ATO, but had no significant effect on Dar-induced apoptosis. We also showed that BRG1 shRNA knocked-down NB4 cells exhibit a lesser HO-1 activation upon ATO treatment, and are more sensitive to ATO-induced apoptosis. We conclude that the lack of HMOX-1 activation is partially responsible for Dar’s enhanced anti-tumor properties.
Impact of Ribavirin on Epithelial-Mesenchymal Transition

Huor, B., Del Rincon, S., Pettersson, F., Zahreddine, H., Borden, K. and Miller, W.H.

The epithelial to mesenchymal transition is a process that can be induced by the transforming growth factor-beta (TGFβ) and closely mimics the steps of metastasis. Our unpublished data has revealed that TGFβ induces an increase in the phosphorylation of the oncogenic protein called eukaryotic translation initiation factor (eIF4E) during EMT. eIF4E acts as a key component in the initiation of protein translation and is overexpressed in numerous human malignancies. Our lab recently published that the drug Ribavirin inhibits the growth of breast cancer cells by targeting eIF4E. Since our preliminary data suggests that Ribavirin may act, in part, by decreasing the phosphorylation of eIF4E in human breast cancer cells, we decided to test the hypothesis that Ribavirin could also antagonize TGFβ-stimulated phospho-eIF4E. If phospho-eIF4E is necessary for promoting EMT, we hypothesize that Ribavirin may be an effective therapeutic to block this process. We have shown that Ribavirin can antagonize molecular and behavioural characteristics of EMT. Specifically, Ribavirin can inhibit (1) migration of TGFβ-stimulated normal mouse mammary epithelial cells (NMuMG), (2) decrease TGFβ-induced MMP9 activity in NMuMG cells, and (3) attenuate the TGFβ-mediated changes in E-cadherin, and Fibronectin levels, epithelial and mesenchymal markers, respectively. We have begun to extend these results to a head and neck cancer model of EMT by testing the impact of Ribavirin on FaDu cells and Ribavirin-resistant FaDu cells.
The Effects of in vivo Sodium Tungstate Exposure on Murine B-Cell Development

Kelly, A., Lemarie, M., Young, Y.K., Eustache, J., Guilbert, C. and Mann, K.K.

High environmental tungsten levels have been associated with a cluster of childhood pre-B acute lymphoblastic leukemia, although the causality between tungsten and leukemogenesis has not been established. Our in vitro data suggest that developing B lymphocytes are susceptible to tungsten-induced DNA damage and growth inhibition. We therefore hypothesized that tungsten could alter the development of B-cells leading to a pro-leukemogenic phenotype. To test this, we exposed C57BL/6J mice over 16 weeks to tungsten-containing tap water at concentrations of 15, 200, and 1000 mg/L, levels that are representative of the environmental concentrations found in the area of the leukemia cluster. Exposure to 1000 mg/L tungsten resulted in a decrease in overall body weight, although mice appeared otherwise healthy and continued to gain weight at a rate similar to other treatment groups. Tungsten exposure, at all tested concentrations, decreased the number of peripheral white blood cells, including monocytes, granulocytes, and lymphocytes. Furthermore, tibia tungsten concentration, as measured by ICP-MS, reached a dose-dependent plateau within 4 weeks. We examined changes in B cell development using flow cytometric analysis and correlated this with tungsten exposure levels. Exposure correlated with an increase in mature B-cells (IgM+, IgD+, B220/CD45R+) within the bone marrow, although no changes in B220/CD45R+ populations in the thymus or the spleen were observed. The activity of B cell progenitors was increased by 4 weeks following tungsten exposure, as measured by preB colony forming assays. Finally, DNA damage, as assessed by COMET assay, was increased in the non-adherent bone marrow populations of exposed animals, with the greatest increase at the lowest tested concentration. Together, these data suggest that tungsten exposure may alter development of B lymphocytes, possibly promoting a pro-leukemogenic phenotype.
Cells Resistant to Vorinostat Show an Increase in ER Stress Markers and Elevated Sensitivity to the Proteasome Inhibitor Bortezomib

Kinal, M. and Dupere-Richer, D.

Histone deacetylase inhibitors (HDACi) are anti-cancer agents that have shown promising activity in hematological malignancies. However, only a small percentage of patients initially respond to treatment and all will eventually develop resistance. HDACi are known to inhibit deacetylation of histones, but it’s crucial to know that they also inhibit deacetylation of various non-histone proteins making the HDACi mechanism of action inherently complex. Revealing mechanisms of HDACi resistance will help elucidate their modes of action, as well as help overcome de novo and acquired resistance in the clinic. To study HDACi resistance in hematological malignancies we developed a vorinostat resistant cell line by a dose escalation protocol from the monocytic-like lymphoma cell line U937. We found that vorinostat resistant cells are very sensitive to the proteasome inhibitor bortezomib and this correlates with an upregulation of some endoplasmic reticulum (ER) stress markers. It was also discovered that the resistant cells have increased proteasome capacity, perhaps to compensate for increased ER stress. ER stress is caused by an accumulation of misfolded proteins in the ER lumen that causes a multifaceted response that is primarily cytoprotective called the unfolded protein response (UPR). The misfolded proteins that are deemed unsalvageable by the ER are targeted for degradation by the proteasome. We hypothesize that increased ER stress could be a mechanism of vorinostat resistance because the UPR induces the upregulation of pro-survival genes. Understanding vorinostat resistance holds clinical relevance in terms of improving HDACi therapy and being able to identify subsets of patients who would benefit most from combination therapy such as vorinostat with bortezomib.
Breakpoint analysis of 65 high-grade serous ovarian carcinomas further indicates that chromosome 19 tends to have more breaks than expected.


High-grade serous ovarian carcinomas (HGSOC) display a high frequency of somatic TP53 mutations and extensive aneuploidy. Though genetic heterogeneity is extensive, non-random patterns of structural anomalies have been described, suggesting that the disrupted regions contain genes important in the etiology of the disease. Previously we have observed a higher than expected rate of chromosomal breaks on chromosome 19 in a small group of eight HGSOC samples. Here we extend our findings to a larger cohort of 65 HGSOC samples to further validate our findings.

Methods: We used ASCAT to characterize genomic anomalies, heterozygosity and chromosomal breakpoints in 65 HGSOC samples that were genotyped using the Illumina Human 610-Quad BeadChip and previously investigated for p53 protein by immunohistochemistry. Results: We fitted a Poisson model for the number of breaks per chromosome, adjusted for chromosome length. The high number of breaks observed on chromosome 19 was highly significant in comparison to all other chromosomes. This observation was independent of P53 protein where 42 of 65 samples were p53 immunopositive where the majority harbored a TP53 missense mutation. Chromosomal breakpoints occurred in or near the predicted transcription domain of 1503 genes, of which 14 genes (found on four chromosomes) were implicated in at least 20 samples tested. Conclusions: Here we bring stronger evidence to warrant the further investigation of chromosome 19 in the identifying genes disrupted in HGSOC.
Arsenic trioxide induces apoptosis through the activation of ASK1 and is enhanced by a thioredoxin reductase inhibitor

Kwan, S., Garnier, N., Miller, W.H. and Mann, K.K.

Arsenic trioxide (ATO) is an effective treatment for acute promyelocytic leukemia (APL). While it is undergoing clinical trials for numerous malignancies including multiple myeloma, myelodysplastic syndrome, lymphoma and solid tumors, it has demonstrated only limited efficacy as a single agent. However, it may hold promise as part of a combination therapy. Thus, investigating the mechanism of action of ATO may lead to generation of rational combination therapies to increase its therapeutic spectrum. Previous work has described the involvement of reactive oxygen species (ROS) in ATO-induced apoptosis as well as the subsequent induction of the specific mitogen-activated protein kinase (MAPK) cascade that includes both stress-activated protein kinase (SAPK)/ERK kinase 1 (SEK1) and c-Jun N-terminal kinases (JNK) activation. However, the link between ROS production and activation of SEK1 remains to be elucidated. Apoptosis signaling kinase 1 (ASK1) is a MAP3K upstream of SEK1 that has been implicated in the induction of stress-induced signaling. Here, we show that ATO activates ASK1 in a dose- and time-dependent manner in an APL cell line. We also show that murine embryonic fibroblasts (MEFs) from ASK1 deficient mice are less susceptible to ATO-induced apoptosis and growth inhibition. Moreover, they exhibit markedly lower ATO-induced JNK activity compared to wild type cells. One model of ASK1 regulation suggests that ASK1 is sequestered in an inactive form by reduced thioredoxin-1 (Trx1). During oxidative stress, Trx1 is oxidized and subsequently releases ASK1 for activation. Immunoprecipitation of ASK1 followed by immuno blotting for Trx1 in APL cells shows a strong basal association that is lost with ATO treatment. Furthermore, the activity of thioredoxin reductase 1 (TrxR1), an enzyme that converts oxidized Trx1 into reduced Trx1, is significantly decreased following ATO treatment. This suggests that ATO activates ASK1 signaling by ROS-mediated oxidation of Trx1 and by inhibiting the restoration of reduced Trx1 by decreasing TrxR1 activity. In addition, we show that inhibition of TrxR1 with the TrxR1 inhibitor Auranofin sensitizes APL cells to ATO-induced apoptosis. This suggests that regulation of ASK1 is dependent on Trx1 redox states. Overall, our results suggest that targeting Trx1 may enhance ASK1 signaling and ATO-induced apoptosis in a novel combination therapy.
SPEN, a novel candidate tumor suppressor gene in breast cancer

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One of the hallmarks of cancer is the accumulation of genetic mutations in tumor cell DNA, resulting in the activation of oncogenes and the loss of tumor suppressor genes. Tumor suppressor genes are genes whose function is to inhibit cancer development by regulating DNA repair, apoptosis, cell growth and proliferation. In the current study, we have investigated the functions of SPEN, which we identified as a novel candidate tumor suppressor gene in breast cancer using an integrated genomic approach. SPEN is known as an estrogen-inducible transcriptional repressor. It localizes to the nucleus where it negatively regulates the transcription of estrogen-responsive genes. This transcriptional repressive effect of SPEN is in part mediated by the recruitment of chromatin remodeling HDACs (Histone Deacetylases) and co-repressors, such as SMRT and N-CoR to Estrogen-Responsive Elements (EREs). Consequently, SPEN is thought to be directly involved in the negative feedback loop attenuating the estrogen-induced hormonal response. To assess tumor suppressive functions of SPEN, we silenced its expression using small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) in the breast cancer cell line MCF-7, which express the 402 kDa protein as confirmed by immunoprecipitation and real-time PCR (RT-PCR). We found that siRNA-mediated suppression of SPEN stimulates MCF-7 cells proliferation by 41% when cells are grown in complete medium. Moreover, consistent with the reported inhibitory effects of SPEN on the estrogen signaling pathway, downregulation of SPEN expression by siRNAs improves cell growth in response to 5 nM estradiol by 35% and increases the transcription of estrogen-responsive genes, such as the progesterone receptors. Because of its role in estrogen response, we investigated whether SPEN expression could have therapeutic consequences by studying the effect of SPEN knockdown on sensitivity of MCF-7 cells to the Selective Estrogen Receptor Modulator (SERM), Tamoxifen. Our results show that stable clones of MCF-7 cells transfected with shRNAs for SPEN are significantly less sensitive to 5 nM Tamoxifen than parental cells. Interestingly, study of a small clinical cohort of ER+/PR+/HER2- breast cancer patients demonstrated that patients with tumors showing low nuclear expression of SPEN had a significantly reduced survival when treated in monotherapy with Tamoxifen. In fact, overall survival of these patients was 50% lower compared to patients whose tumors express high levels of the protein, indicating a role for SPEN in predicting response and survival of patients treated with Tamoxifen. Finally, we determined the prevalence of SPEN mutations in 23 ER+/PR+/HER2- breast cancer samples showing loss of heterozygosity at chromosome 1p, where SPEN is located. (ABSTRACT TRUNCATED AT 400 WORDS).
Effects of targeting g-quadruplex structures in cancer cells

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Intact chromosome ends (telomeres) are required for cancer cell proliferation and survival. Telomeric integrity is maintained by shelterin protein and, most commonly, by telomerase. Loss of telomeric integrity or extensive shortening activates DNA damage checkpoints, leading to cell senescence or death. Detected in ~85% of tumor cells, telomerase is an attractive target for anti-cancer therapy (1). However, targeting telomerase poses several challenges or limitations. An alternative approach is to disrupt telomeres directly, altering the interactions between telomeres and their binding proteins. G-quadruplex ligands stabilize structures arising from the folding of single-stranded G-rich 3'-ends of the telomere, known as the G-quadruplex, which cannot be elongated by telomerase. Stabilization of these structures can mediate rapid antiproliferative effects in cancer cells. The rapid effects observed with certain G-quadruplex ligands appear to be restricted to cancer cells, suggesting that the telomere’s protective nucleoprotein cap may differ in cancer cells versus normal cells (2). Moreover, G-quadruplex structures have been identified in the promoter regions of important protooncogenes (3). Thus G-quadruplex ligands may possess significant therapeutic value, conferring anti-cancer effects via telomerase inhibition, disruption of telomere integrity, and through oncogenic suppression. We hypothesize that two phenanthroimidazole platinum (II) complexes, PIP and CLIP, shown to inhibit telomerase in vitro (4-6) at 0.46±0.29µM and 5.71±1.55µM respectively, stabilize G-quadruplexes and mediate antiproliferative effects in cancer cells. Ligand concentrations causing 50% cytotoxicity were determined in telomerase-positive, in telomerase-negative ALT, and in non-cancerous primary cells. Multiple ligand concentrations were used in a seeding assay to assess the effects of treatment on long-term cell growth, where HUH7 cells treated with PIP and with CLIP showed a decrease in cell growth rate when compared to treatment with the control ligand, BPY. These studies will determine antiproliferative effects of G-quadruplex ligands in cancer cells, will elucidate their mechanism of action, and will validate the development of novel and specific therapeutic ligands targeting G-quadruplex structures. (1) Harley CB. 2008. Nat Rev Cancer 8:167-79. (2) Riou JF. 2004. Curr. Med. Chem. Anticancer Agents. 4, 439. (3) Brooks et al. 2010. FEBS Journal 277, 3459-3469. (4) Kieltyka R et al. 2008. Journal of the American Chemical Society, 130, 10040-41. (5) Kieltyka R et al. 2008. Chemistry - A European Journal, 14, 1145-1154. (6) Castor K et al. 2012. ChemMedChem Jan 2;7(1):85-94
Targeting eIF4E signaling in combination with Fluradabine in CLL

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Chronic lymphocytic leukemia (CLL), the most common leukemia in adults in the western world, is an incurable disease and even though the patients initially respond to available treatments – Rituximab, Fludarabine and cyclophosphamide – the majority develop resistance and relapse. The mammalian target of Rapamycin (mTOR), a downstream effector of AKT, has been identified as a drugable target in haematological malignancies, including CLL. Nevertheless, mTOR inhibitors failed to induce significant apoptosis of either cycling or quiescent cells and have shown so far modest clinical responses associated with toxicity. We present here another approach to target the mTorc-1 downstream target, the translation factor eIF4E. We used the MTT assay to test the viability of the cells after 72hrs of treatment and the annexin V (AV) assay to assess apoptosis after 48hrs. Ribavirin sensitized B-CLL lymphocytes to FLU, decreasing FLU IC50 more than 2 folds. In addition, 4 out of these 7 patients were unmutated for IgVH, corresponding to patients with a worst prognosis. We also assessed the apoptosis induction of the combinations of FLU and ribavirin in cultured media and on cultured media with monolayer bone marrow stromal cells (BMS-2). Ribavirin increased FLU-induced apoptosis on samples tested in BMS-2 cells co-culture condition and in fewer samples when cells were cultured in RPMI conventional media 48hrs after treatment. Together, MTT and AV results on BMS-2 supported media showed that mTor downstream signalling pathway might be a good target in CLL irrespectively of IgVH status for what we suggest extending the studies.
Semi-Synthesis of ubiquitinated androgen receptor peptides using expressed protein ligation

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The Androgen receptor (AR), a steroid member of the nuclear receptor superfamily, primarily functions as a DNA-binding transcription factor to regulate gene expression. AR also has a role in the development of the male phenotype: in fact, AR is activated by binding cytoplasmic testosterone (T) or dihydrotestosterone (DHT) hormone before translocating to the nucleus. Introduction: The 90-kb AR gene is found at locus Xq11-12, which is transcribed to form an mRNA 10.6 kb in length. Eight exons of the AR gene code for AR, a protein of about ~919 amino acids, which has three conserved NR superfamily domains: exon 1 encodes the transactivational domain (TAD); exons 2 and 3 encode the DNA-binding domain (DBD); and exons 4 through 8 encode the ligand-binding domain (LBD). The polyglutamine tract, a continuous stretch of glutamine (Gln) residues, is located in the TAD, and its expansion to 38 or more Gln causes spinal and bulbar muscular atrophy (SBMA). SBMA is an X-linked recessive neurodegenerative disease that is considered to be an polyglutamine (polyGln) expansion disorder. In unaffected individuals, 9-33 CAG repeats which code for Gln are found in exon 1, while males who inherit more than 37 CAG repeats will develop SBMA. The toxic gain of AR function characteristic of SBMA results in muscle weakness, fasciculations, cramping, and wasting; a loss of function is related to breast enlargement and reduced fertility. Ubiquitin-proteasome system (UPS) components are found in polyGln-expanded protein aggregates. The ubiquitin-proteasome system (UPS) degrades proteins involved in metabolic or regulatory pathways in the cell. Aims of the Study: The influence of polyglutamine tract on the protein degradation role of the ubiquitin-proteasome system will be explored. Also, we want to determine if the polyGln-expanded AR can affect the UPS directly. Result: IMPACT-TWIN (Intein Mediated Purification with an Affinity Chitin-binding Tag-Two Intein) was used to clone 0QAR, 20QAR, and 50QAR in (PTWIN2 vector) via PCR and was analyzed using gel electrophoresis. IMPACT-TWIN has the ability to isolate native recombinant proteins possessing a reactive C-terminal thioester in a single chromatographic step without the use of exogenous proteases. These reactive groups can be used in Intein-mediated Protein Ligation to attach peptides or proteins to the C-terminus or N-terminus of a target protein. AR from 0QAR, 20QAR, 50QAR and HUB clones was purified using chitin beads and cleaved using MESNA. Purified samples and cleaved samples were analyzed on SDS-PAGE gel. (ABSTRACT TRUNCATED AT 400 WORDS).
Protein arginine methyltransferase 6 (PRMT6) is an enzyme that catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to its target substrate on an arginine residue. Originally found to play a role in HIV replication and in DNA damage repair by respective methylation of viral Tat protein and polymerase β, recent studies have uncovered that PRMT6 also regulates gene expression by methylation of histone tails. More specifically, PRMT6 can asymmetrically dimethylate histone 3 on arginine 2 (H3R2me2a). This H3R2 methyl mark impairs gene-activating H3K4 methylation by preventing the binding of the MLL methyltransferase complex at promoter regions, thus leading to transcriptional silencing. To investigate physiological roles for PRMT6, we cultured mouse embryonic fibroblasts (MEFs) isolated from PRMT6 knock-out embryos. We found that PRMT6-/− MEFs are prone to premature senescence. This cellular state is characterized by a G0/G1 cycle arrest as a mechanism to protect mammalian cells from uncontrolled growth. It can be triggered by different stress conditions such as DNA damage, oxidative stress, telomere shortening and even activated oncogenes. In mouse cells, hallmarks of senescence include distinctive cell flattening, increased senescence-associated β-galactosidase activity (SA-β-Gal), and upregulation of the ARF-p53 pathway, of cyclin-dependent kinase inhibitors such as p21 and of other markers including PML bodies. These MEFs also show a global hypomethylation of H3R2 histone mark and an upregulation of senescence markers and p53 target genes. Using p53 and PRMT6 knock-out MEFs, we demonstrated that the senescent phenotype caused by a lack of PRMT6 is overruled by depletion of p53. In accordance with these findings, we investigated the role of PRMT6 in the regulation of p53 responsive genes by chromatin-immunoprecipitation (ChIP). Interestingly, we observed a decrease in the repressive histone arginine dimethylation as well as a concomitant increase in activating marks such as H3K4 trimethylation in the Trp53 promoter region of PRMT6-/− MEFs. We also identified downregulation of activating arginine methylation at other p53 responsive genes, leading to transcriptional repression and causing a cell cycle arrest. Finally, we noticed relatively low p53 protein levels in cancer cells that overexpress PRMT6, unravelling an important function for PRMT6 in the regulation of the p53 tumor suppressor. Triggering cellular senescence by targeting PRMT6 in order to reverse its antagonizing role on p53 may be an interesting avenue in cancer therapy. This work is supported by FRSQ and CIHR.
Combination therapy with the histone deacetylase inhibitor panobinostat and the CD20 targeting antibody rituximab in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common sub-type of non-Hodgkin’s lymphoma and although potentially curable with combination chemotherapy (CHOP-R), it remains a therapeutic challenge in patients who do not respond. Recent work has demonstrated frequent mutations in histone modifying enzymes in DLBCL, particularly in histone acetyl transferase genes. We therefore, speculated that perturbations of epigenetic marks play a driving role in some of these cancers and that treatment with a histone deacetylase inhibitor (HDACi) might normalize acetylation levels and thus, alleviate the oncogenic potential of targeted cells. Purpose of the study: Several clinical trials have shown that HDACis as single agents are only effective in a minority of patients. We therefore, sought to find combination treatments to enhance the anti-cancer effects of HDACis. Herein, we describe our results investigating the combination therapy of the HDACi panobinostat (LBH-589; LBH) and the CD20 targeting antibody rituximab in DLBCL. Methods: Six DLBCL cell lines were treated with LBH and/or rituximab in vitro for 48 hours at concentrations tolerable in humans. Cell death and cell viability were measured by assay of DNA content by propidium iodide stain using flow cytometry, and manual cell counting using Trypan blue, respectively. No complement or immune effector cells were added to ensure that only direct signaling effects of rituximab binding to CD20 were measured. CD20 cell surface expression was measured by flow cytometry. Synergy of combination treatment was calculated using CalcuSyn software. Protein expression was assessed by western blot. In parallel with the in vitro studies, a multi-center, phase II clinical trial investigating LBH+-/- rituximab is being conducted. Correlative patient samples will be used to verify whether mechanisms of synergy observed in cell lines can be translated to human beings. Results: In four out of six DLBCL cell lines, we see a synergistic increase in cell death with the combination of LBH and rituximab. The effect of rituximab alone on cell death is negligible in all but one cell line, indicating that rituximab signaling on its own is not sufficient to induce cell death. In contrast, rituximab does sensitize four out of six cell lines to LBH-induced cell death. We find that HDACi treatment did not increase cell surface expression of CD20, but rather CD20 expression remained unchanged or was reduced. Despite this, synergy between LBH and rituximab is still observed. No synergy was seen in a CD20-negative multiple myeloma cell line, suggesting that CD20 expression is necessary to transduce the signal from rituximab that sensitizes to HDACi treatment. (ABSTRACT TRUNCATED AT 400 WORDS).
The role of p66Shc in mammary tumorigenesis

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ShcA has been demonstrated to be a key signaling molecule in the regulation of survival, angiogenesis, immune suppression and metastasis in breast cancer cells. However, the role of p66Shc, one of the ShcA isoforms, in tumourigenesis is poorly understood. The anti-tumorigenic properties of p66Shc results from its induction of oxidative stress upon translocation into the mitochondria, where it catalyzes electron transport and reactive oxygen species (ROS) formation, leading to apoptosis. However, ROS can also contribute to tumour initiation and progression, and pro-tumorigenic properties of p66Shc have been observed. Our objective is to determine whether p66Shc confers pro- or anti-tumorigenic properties during breast cancer progression. We aim to achieve this by characterizing the impact of p66Shc expression on mammary tumourigenesis both in vitro and in vivo. To this end, an ErbB2-driven breast cancer cell line (NOP 21), low in endogenous p66Shc levels, was engineered in our lab to stably overexpress p66Shc. In vitro, initially after transfection, NOP 21 p66Shc-overexpressing (p66) cells showed an increased stress response signaling and ROS levels, as well as decreased proliferation and survival signaling compared to NOP 21 vector control cells (VC). Since we observed that p66 cells significantly increased angiogenesis in matrigel plug experiments, and it is known that high ROS mimics hypoxia, we attempted to characterize regulators and downstream effectors of hypoxia in p66 cells. Though Hif1a was better stabilized in these cells, VEGF expression and reliance on glycolysis, both normally up-regulated in the presence of Hif1a, were both surprisingly found to be significantly decreased. We plan to further characterize the effect p66Shc overexpression has on angiogenesis and alternative methods of energy metabolism. Finally, in our aim to characterize the in vivo implications of p66Shc in mammary tumourigenesis, we recently injected VC and p66 cells into the mammary fat pads of nude mice and will observe the effects on tumour growth, proliferation, angiogenesis, hypoxia, apoptosis and metastasis. In conclusion, these studies will help gain insight into the dual role of p66 in tumourigenesis and therefore are important as they may potentially form the basis for novel cancer therapeutics.
Targeting the Apoptotic and Autophagic Pathway to Augment Oncolytic Virotherapy of Chronic Lymphocytic Leukemia

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Oncolytic viruses (OVs) can selectively infect and kill cancer cells, while leaving normal cells and tissues largely unaffected. Vesicular stomatitis virus (VSV) is a prototypical OV that exploits defects in interferon and antiviral signaling pathways to replicate in and kill tumor cells. However, many primary cancers, including chronic lymphocytic leukemia (CLL) are largely resistant to VSV-induced cell lysis due to overexpression of the anti-apoptotic members of the Bcl-2 family. We have shown that resistance of CLL to VSV-induced oncolysis can be overcome using a combination of VSV and small molecule Bcl-2 inhibitors, such as GX15-070 and ABT-737 that disrupt protein-protein interactions between the anti-apoptotic Bcl-2 proteins and pro-death proteins (Samuel et al, Mol. Ther. 2010), thus identifying a mechanism of synergistic cell death. Bcl-2 family members are also implicated in the regulation of autophagy, in part through their inhibitory interaction with Beclin1 - a central mediator of autophagy. In the present study, we expand our previous findings to investigate the ability of the VSV/Bcl-2 inhibitor combination to induce autophagy as a means of cell death in CLL. We hypothesize that pharmacologic disruption of the Bcl-2/Beclin1 interaction will stimulate autophagy and sensitize cancer cells to cytotoxic treatments using VSV. Co-immunoprecipitation studies in leukemic cell lines revealed that pro-survival proteins form inhibitory interactions with Beclin1. The combination of GX15-070 and VSV was synergistic in reducing cell viability, disrupting inhibitory interactions of Beclin1 with Bcl-2 and Mcl-1, as well as increasing Beclin1 protein levels, thus biasing the cells toward autophagy. In primary ex vivo CLL patient samples, cell death caused by VSV/ABT-737 could be reversed by the late stage autophagy inhibitor chloroquine. VSV, in combination with ABT-737, induced LC3-II accrual and p62 degradation, both indicators of autophagic flux. Furthermore, inhibiting autophagy at an early stage using 3-methyladenine, led to increased apoptosis in CLL samples. Our data provides new insights into the mechanism of cell death induced by viral oncolysis in combination with Bcl-2 inhibitors. Targeting of Beclin1 by Bcl-2 members represents an important mechanism of autophagy regulation, and highlights possible crosstalk between the apoptotic and autophagic pathways.
Identification of MAFF target genes in myometrial cells:
Relevance to inflammation and preterm labor

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The MAFF transcription factor, a member of the MAF transcription factor family, is a basic leucine zipper protein. It binds to MARE consensus sequences as a homodimer or heterodimer, together with the CNC transcription factors, to either activate or repress gene expression. We have previously shown that proinflammatory cytokines interleukin 1-beta (IL1B) and tumor necrosis factor (TNF) induce MAFF transcript and protein levels in myometrial cells. Evidence suggests that there is a clear link between cytokine signalling, inflammatory response and preterm birth. IL1B and TNF have been implicated in human parturition, and have also been associated with preterm labor, particularly related to intrauterine infection. Moreover, MAFF has been found to be highly expressed in uterine smooth muscle at term. Therefore, we have knocked-down MAFF expression by small interfering RNA in hTERT-C3 myometrial cells. We will compare gene expression in myometrial cells in presence or absence of the MAFF transcription factor, as well as IL1B, to determine the genes regulated by MAFF in myometrial cells at term. We expect these results to shed light on the functional role of rapidly induced MAFF transcription factor in uterine smooth muscle cells and to help elucidate the mechanisms related to inflammation in preterm birth.
FEN1 is a target of the SET7 lysine methyltransferase during DNA replication

Thandapani, P. and Richard, S.

Lysine methylation is a post translational modification that regulates the DNA damage response pathway. To identify new lysine methylated proteins, a peptide array encompassing potential lysine methylation sites from over 100 key DNA damage response proteins were incubated with lysine methyltransferase SET7. We identified Flap endonuclease 1 (FEN1) as a substrate of SET7. FEN1 is a structure specific endonuclease with important functions in Okazaki fragment processing during DNA replication. FEN1 knockout mice are embryonically lethal and mutations that affect FEN1’s enzymatic activities have been indentified in human cancer specimens. We hypothesize that methylation could potentially regulate the functions of FEN1. To test this hypothesis, we first mapped the lysine residue methylated by SET7 using biotinylated peptides. The specific residue methylated by SET7 was further confirmed by mass spectrometric analysis. We verified the endogenous methylation of FEN1 by generating a methyl specific antibody. K-R mutation of the specific lysine residue abrogated the signal detected by the methyl specific antibody. Furthermore, knockdown of the methyltransferase SET7 using siRNAs reduced the levels of FEN1 methylation as detected by immunoblotting with our methyl specific antibody. This confirmed SET7 as the physiological enzyme methylating FEN1 at the specific lysine residue. Since FEN1 has important functions in DNA replication and is highly active during S phase of the cell cycle, we hypothesised a possible regulation of FEN1 methylation during cell cycle progression. To test this hypothesis, we arrested cells at G1/S using hydroxyurea and followed FEN1 methylation along the cell cycle. FEN1 methylation increased as cells progressed through S phase and decreased as they exited S phase. This increase in methylation during S phase was found to be SET7 dependent as knockdown of SET7 reversed this increase in methylation. We have also identified PHF20L1 to be a methyl specific interactor of FEN1. We are currently exploring if methylation affects FEN1’s enzymatic activities and the functional significance of the methyl specific interaction of PHF20L1. Conclusion Flap endonuclease 1, a structure specific endonuclease involved in the processing of Okazaki fragments and base excision repair is modified by lysine methyltransferase SET7. Methylation of FEN1 likely modulates its activities during DNA replication.
Genetic Factors in Non-epithelial Ovarian Cancer


Germ cell tumours (GCTs) often arise in younger men and women as testicular and ovarian GCTs (TGCTs and OGCTs), respectively. While in young men TGCTs are often malignant, in young women benign OGCTs are more common, making up ~20% of all ovarian abnormalities. However, a rare sub-set are malignant OGCTs (mOGCTs), accounting for <5% of all ovarian cancers. GCTs contribute to the 10% of non-epithelial forms of ovarian cancer, along with Sertoli-Leydig Cell Tumours (SLCTs), granulosa cell tumours, sex cord stromal tumours, and sarcomas. Different types of GCTs include dysgerminomas, embryonal carcinomas, yolk sac tumours, teratomas and mixed GCTs, composed of various tissue types. While mOGCTs are often curable, they have a worse prognosis than TGCTs.

Though it is rare, several cases have been documented describing families with more than one relative affected with mOGCT and/or TGCT. Potential genetic susceptibility factors have been found in TGCTs; therefore, the familial incidence of mOGCTs, along with genetic associations in other types of ovarian tumours, led us to believe that susceptibility genes in mOGCTs exist and that some of the cases are caused by either deleterious mutations in one or more single genes, or by associations between mutations in several genes.

We collected DNA samples from GCTs in 5 of the 8 reported OGCT/TGCT families, with one more family’s samples pending. We did whole exome sequencing in 3 families, and found what are predicted to be deleterious indels, nonsense, missense and splice site mutations in several candidate genes, including OVGP1, SMARCA4, CCDC110, GSX1, CEP250, TP53BP1, ZFAND1 and WSCD1. As well, we collected 161 sporadic OGCT DNAs and 31 of the patients’ germline DNAs, and are now working through sequencing the exons of these genes, starting with SMARCA4 and OVGP1. We also sequenced the RNAse IIIa and IIIb domains of DICER1, as mutations in this gene have been found in these domains in SLCTs and GCTs.

We have so far found 1 missense mutation in OVGP1, 4 missense and several synonymous and intronic mutations in SMARCA4.
DICER1 RNAseIIIb domain is mutated in two different ways in two different lesions in the same patient


DICER1 is an endoribonuclease responsible for the generation of microRNAs (miRNAs). miRNAs are responsible for the repression of many genes. DICER1 has been found to be mutated in pleiotropic tumour predisposition syndrome (OMIM 601200). Families with germline mutations in DICER1 are at increased risk of developing a variety of tumours associated with pleiotropic tumour predisposition syndrome. Here we present a patient who presented with multinodular goiter and Wilms tumour. We discovered that the proband inherited one germline DICER1 mutation and acquired different somatic mutations in DICER in her Wilms tumour and in her goiter. These data are consistent with a variant of the classical two-hit model of tumour formation, in that the first hit is likely inactivating whereas the second hit is highly specific. These data support the hypothesis that DICER1 is a novel type of tumour suppressor gene.
5'pppRNA activates RIG-I-mediated antiviral and inflammatory responses against H1N1 Influenza Virus


With new antigenic strains of influenza continuing to emerge, rapid generation of effective vaccines remains a challenge, and antigen-independent prophylaxis may provide an alternative approach to enhance host resistance against infection. The characterization of agonists that potently activate the innate immune system, trigger the interferon (IFN) antiviral response and inhibit H1N1 Influenza virus infection are potentially useful in limiting virus multiplication in vivo and may also serve as adjuvants in vaccine development. The cytosolic RIG-I pathway is activated by many RNA viruses - including influenza - via viral RNA replicative intermediates that contain short hairpin dsRNA and 5’ triphosphate structures. We designed RIG-I agonists based on sequences from the 5’UTR regions of distinct negative-strand viruses - VSV, Influenza, Rabies, Measles, and Sendai virus and demonstrated that all RIG-I agonists activated the RIG-antiviral response at concentrations in the picomolar range. The antiviral effect was demonstrated in human bronchial epithelial A549 cells, where treatment with 5’pppRNA potently induced IRF3 phosphorylation and dimerization, as well as a >100-fold increase in the transcription of interferon stimulated genes (ISGs) and genes involved in inflammation. Furthermore, 5’pppRNA pre-treatment dramatically blocked virus replication in A549 cells challenged with H1N1 A/PR/8/34 Influenza virus. The magnitude and duration of ISG and inflammatory gene expression was further evaluated by Illumina gene expression profiling, where 5’pppRNA triggered a rapid, sustained and diverse range of antiviral and inflammatory genes compared to treatment with IFN?. Further bioinformatics analysis based on available datasets also characterized distinct subsets of IRF3 or IRF7 activated genes. Intranasal delivery of 5’pppRNA to BALB/c mice demonstrated the induction of an antiviral response in mouse lungs and resistance to influenza upon virus challenge. These results illustrate that naturally derived RIG-I agonists block early stages of influenza infection and represent a potent stimulator of the innate antiviral response.