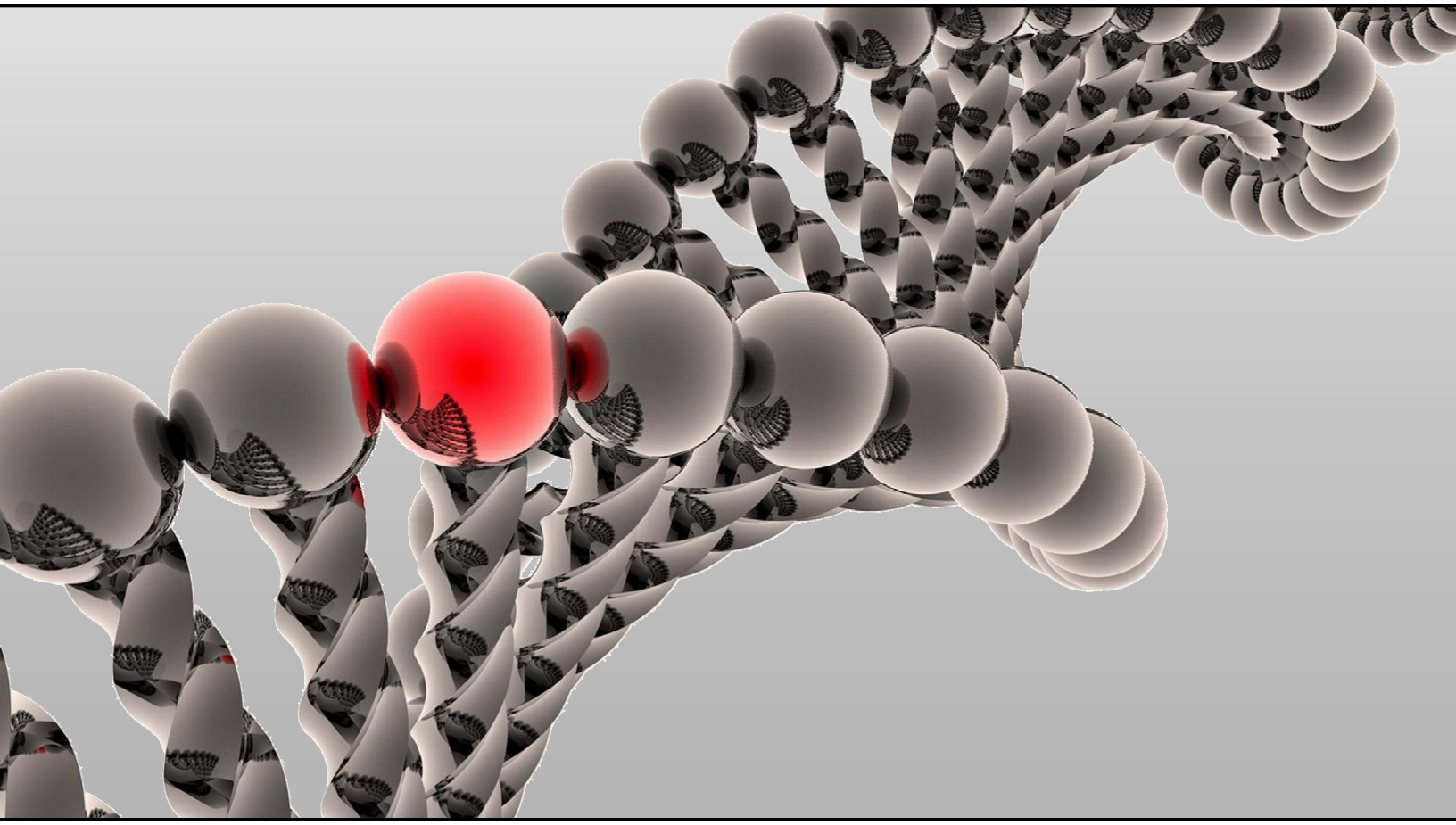
The **Southern Alberta Cancer Research Institute**
proudly presents the first annual

CALGARY CANCER RESEARCH SYMPOSIUM 2015

Friday, January 30th at *MacEwan Conference & Events Centre,* University of Calgary, Alberta



UNIVERSITY OF CALGARY

Alberta Health Services

SOUTHERN ALBERTA Cancer Research Institute

PROGRAM

FRIDAY, January 30th, 2015

MacEwan Conference & Events Centre, University of Calgary

8:00 – 9:00am **ARRIVAL AND REGISTRATION**
(*Continental Breakfast (assortment of fresh baked goods)*

9:00 – 9:10am **WELCOME AND INTRODUCTIONS:**
Dr. Greg Cairncross, SACRI Director

9:10 – 9:55am **KEYNOTE ADDRESS: Dr. Sam Weiss,** Director, Hotchkiss Brain Institute. *"Brain Tumour Stem Cells and STAT3 Oncogenic Signalling in GBM"*

10:00 – 10:20am Morning BREAK (*Fresh and savory snacks)*

- 10:25 – 10:50am **Dr. Ivo Olivotto**
  "*Lessons Learned from Randomized Trials of Radiotherapy for Breast Cancer*"
- 10:55 – 11:20am **Dr. Christine Friedenreich**
  "Physical Activity in Cancer Control: Biologic and Epidemiologic Evidence
- 11:25 – 11:50pm **Dr. Linda Carlson**
  "*Integrative oncology at the Tom Baker Cancer Centre: Program Development and Evaluation" Is this possible?*"

11:55 – 12:40pm Lunch (*Custom Chef's Lunch Buffet)*

- 12:45 – 1:10pm **Dr. Steve Robbins**
  "*A Basic Scientists Unexpected Journey into Translational Medicine*"
- 1:15 – 1:40pm **Dr. Nizar Bahlis**
  "*Genomic Studies Unveil the Mechanisms of Resistance to IMIDs in Myeloma"*
- 1:45 – 2:10pm **Dr. Paula Robson**
  "*Alberta's Tomorrow Project - Making the Transition from Cohort Study to Health Research Resource*"
- 2:15 – 2:40pm **Dr. Alain Tremblay**
  "Lung Cancer Screening in Alberta"

2:45 –3:05pm	Afternoon BREAK *(Fresh and savory snacks)*
• 3:10 – 3:35pm	**Dr. Susan Lees-Miller** *"Mechanisms of Genome Instability"*
• 3:40 – 4:05pm	**Dr. Greg Cairncross** *"Defining a role for PARP inhibition in the treatment of GBM"*
4:10 – 5:45pm	**POSTER SESSION** (*Wine and Cheese Reception)*
6:00 – 8:00pm	**BANQUET DINNER** (Pre-Registration Required) *(Custom Plated Dinner Menu)*

SOUTHERN ALBERTA
Cancer Research Institute

Dear SACRI Members,

Through a generous donation by the Muller Family. We are very excited and pleased to welcome you to the 2015 Southern Alberta Cancer Research Institute Research Symposium.

The goal for today is to connect with one another, socialize and learn about the exciting new science emerging from all disciplines of the Southern Alberta Cancer Research Institute.

SACRI is very delighted to have Dr. Sam Weiss present our keynote address "*Brain Tumour Stem Cells and STAT3 Oncogenic Signalling in GBM*". In addition, we have invited nine further speakers selected from SACRI's many scientific leaders, and have aimed as much as possible to represent the many different facets of SACRI's work for us all to enjoy.

It is of great pleasure that we are able to offer our trainees the opportunity to present their work during the poster session, for which cash prizes (gold, silver, bronze level) will be awarded for the top three outstanding presentations.

With warmest regards,

Dr. Aaron Goodarzi and Ms. Carmen Coelho

Our 2015 Keynote Speaker

Dr. Sam Weiss is a Professor in the Cumming School of Medicine's Departments of Cell Biology and Anatomy and Physiology and Pharmacology at the University of Calgary. An Alberta Heritage Foundation for Medical Research Scientist, he received his BSc in Biochemistry from McGill University and his PhD in Neurobiology from the University of Calgary. He is the inaugural and continuing director of the Hotchkiss Brain Institute at the University of Calgary whose mission is to translate innovative research and education into advances in neurological and mental health care. The Hotchkiss Brain Institute is now a world class research institute of 450 scientists and trainees, along with 300 professional staff.

Dr. Weiss' own explorations into the brain have changed the fields of developmental neurobiology and neural regeneration, and have earned him one of the world's most prestigious medical science awards, a Gairdner International Award. Dr. Weiss was elected a Fellow of the Royal Society of Canada in 2009, and a Fellow of the Canadian Academy of Health Sciences in 2014.

In 1985 (Nature 371:717), together with Dr. Fritz Sladeczek, Dr. Weiss discovered the metabotropic glutamate receptor - now a major target for pharmaceutical research and development for neurological disease therapies. In 1992 (Science 255:1707), Dr. Weiss discovered neural stem cells in the brains of adult mammals. The groundbreaking discovery that dispelled dogmas that the brain cannot regenerate, has led to new approaches for brain cell replacement and repair. Dr. Weiss's current research focuses on transformed brain stem cells that give rise to malignant gliomas. It is hoped that studies of the comparative distinctions of neural and glioma stem cells will shed new light on how to treat the currently untreatable malignant gliomas.

Dr. Weiss has authored nearly 100 publications, holds over 20 patents in the neural stem cell field and has founded three biotechnology companies in this area. He sits on numerous national and international peer review committees. He currently chairs the International Scientific Advisory Boards of the NeuroDevNet Network of Centres of Excellence (UBC) and the McMaster Stem Cell and Cancer Research Institute, as well as the International Research Advisory Board of the University Health Network (Toronto).

Poster Abstracts

01 - Oncolytic Reovirus Improves Immunotherapeutic Strategies for Breast Cancer

Ahmed Mostafa, Jason Spurell, Qiao Shi, Kathy Gratton, and Don Morris. Department of Oncology, Tom Baker Caner Center, University of Calgary, Calgary, Alberta

Background: Breast cancer is the most common cancer affecting Canadian women and accounts for nearly 30% of all newly diagnosed cancer cases. Despite a large choice of treatments it is still difficult to predict the outcome of breast cancer and the response to treatment. Thus, better treatment strategies are needed especially in patients with poor prognosis. Oncolytic viruses such as reovirus (RV) are non-pathogenic viruses, which specifically target and lyse cancer cells due to genetic abnormalities, with no effect on normal cells. Recently, RV is used in human phase III clinical trials against different histological malignancies. The challenge of these trials is the elicitation of anti-viral immune response, which results in viral clearance. Hypothesis: We hypothesized that RV, in addition to its direct oncolytic effects can be used as an immune adjuvant for both augmentation of immune effector mediated tumour regression and immune surveillance against breast cancer recurrence. Experimental Designs: Murine dendritic cells (DC) from Balb/c were propagated and pulsed with EMT6 cells infected with RV to examine weather functional immune effectors could be generated in vitro. In vivo studies were done using a syngenic murine breast cancer model (EMT6/Balb/c). Results: We have demonstrated that RV infected breast cancer cells is an efficient priming agent for DCs. Moreover, using in vitro LDH cytotoxicity assays these DCs are capable of anti-tumour activity. In order to prove that these effectors had activity in vivo we immunized mice with either s.c injection of EMT6/RV (SC group) or i.v injection of DCs pulsed by EMT6/RV (IV group). The SC group had significantly reduced tumor recurrence compared to control group. Interestingly IV group were completely protected from tumour recurrence. In turn, we also identified that IV group had the highest percentage of CD4+ and CD8+ T cells with higher cytotoxic activity and T cell proliferation to tumour antigen compared to the other groups. Significance: To the best of our knowledge, the idea of combining RV, and DC have never been tried in treatment of cancer. Taken together, these data will provide new treatment strategies with resultant improved efficacy and safety of our breast cancer patients to be translated into phase I/II clinical trials.

02 - Investigation of natural products from Canadian prairie plant species *Thermopsis rhombifolia* for anti-cancer activity by phenotypic assays

Alessandra Bosco, Kernéis-Golsteyn, Sophie, and Golsteyn, Roy M. Cancer Cell Laboratory, University of Lethbridge, Department of Biological Sciences, Lethbridge, AB, Canada

Among the current chemotherapies used to treat cancer, almost 50% of them are either natural products or their derivatives. One class of natural products, known as secondary metabolites, are a particularly valuable source of chemical entities with important biological activities. We have launched the Prairie to Pharmacy Program in which we investigate endemic plant species from the prairie ecological zone for anti-cancer activities. Many plant species from the prairies have not been previously investigated. It is likely that they harbour secondary metabolites as defences to herbivory by mammals and insects, as well to abiotic stressors.

The plant species *Thermopsis rhombifolia*, locally known as the buffalo bean is endemic to the prairie ecological zone. It was reported to be toxic to children by accidental ingestion in a clinical case report. We used a series of phenotypic screens to investigate the anti-cancer properties of an ethanolic *T. rhombifolia* extract. Extract PP-003 was cytotoxic to the human cancer cell lines HT-29 (colon) and to SH-SY5Y (brain) at IC50 concentrations of 180 ug/mL and 200 ug/mL, respectively, but it was not cytotoxic to the normal (non-cancerous) human WI-38 cell line (IC50 >1000 ug/mL). HT-29 cells treated with extract PP-003 arrested in the G1 phase (84% of total cell population) of the cell cycle with only 2% of the cells in S-phase (Figure 2). This effect was not due to a genotoxic activity, as a DNA damage signal (histone gamma H2AX) was not observed after extract PP-003 treatment. We found by western blotting that cancer cells treated with extract PP-003 contained little Mcl-1 protein. Furthermore, cells treated with extract PP-003 were strongly adherent in a cell binding assay. We are collaborating with the pharmaceutical company Pierre Fabre Laboratories (France) to isolate the active compound by biology-guided fractionation. Several other plant species, such as *Gaillardia aristata* also have shown anti-cancer activities in preliminary tests.

This project has been made possible through grants from the University of Lethbridge Research Fund.

03 - Regulatory Mechanisms of Epithelial-Mesenchymal Transition

Amrita Singh, Shoraf Dadakhujaev, Stuart Netherton, Lili Deng, Shirin Bonni
Department of Biochemistry & Molecular Biology and Southern Alberta Cancer Research Institute and, Cumming School of Medicine, University of Calgary, Calgary, Alberta.

Epithelial-mesenchymal transition (EMT) is a fundamental cellular process in development, and can be reactivated in cancer contributing to tumor invasiveness and metastasis. The transforming growth factor beta (TGFβ) is a key inducer of EMT, but the mechanisms that regulate TGFβ-induced EMT remain incompletely understood. We are interested in examining the role and regulation of the ubiquitin pathway in controlling EMT. Our research has significant implications for our understanding of the molecular mechanisms underlying processes in development and cancer.

04 - A combination of MEK inhibitor cobimetinib and cis-retinoic Acid induced differentiation and decreased viability in neuroblastoma cell lines

Anjali Singh, MSc and Aru Narendran, MD, PhD

Neuroblastoma is the most common extracranial solid pediatric tumor and currently, high-risk cases of NB carry extremely poor long-term survival rates. The RAS/MAPK pathway has been shown to be involved in neuroblastoma (NB) tumorigenesis. We hypothesize that RAS/MAPK pathway inhibition via MEK inhibition would be effective against NB growth stimulatory mechanisms. Cobimetinib is a MEK inhibitor currently in clinical trials as an anticancer agent for the treatment of BRAF-Mutated Melanoma. Using cell lines (n=6), representing the spectrum of molecular abnormalities seen in specimens obtained from NB patients, we have evaluated the effects of cobimetinib in preclinical and target modulation studies. In these assays, cells were exposed to cobimetinib, either alone or in combination with cis-retinoic acid, an agent that has been shown to induce differentiation in NB cells. Our results show that cobimetinib alone induced a concentration-dependent reduction of cell viability. Out of the cell lines tested, IMR-32 was most sensitive for cobimetinib with an IC_{50} value of less than 0.1uM. SHEP, SK-N-SH, SK-N-BE(2) and SK-N-AS lines showed intermediate activity with IC_{50} values 0.1 and 1 uM while IMR-5 was found to be most resistant. To correlate the MEK1/2 activity with NB cells sensitivity, we analyzed the expression of MEK1/2 phosphorylation in all NB cell lines. Cells with high expression of phosphorylated MEK1/2 were more sensitive compared to those with lower expression. In addition, cobimetinib showed feedback activation of MEK1/2, and dephosphorylation of c-RAF and ERK1/2 kinase. The differentiation markers, GFAP and MAP2 were analyzed using immunofluorescence. The NB cells treated with cobimetinib alone and in combination with cis-retinoic acid were positive for differentiation markers. Thus cobimetinib alone was also able to induce differentiation, which was enhanced after combined treatment with cis-retinoic acid. Collectively, our results suggest that MEK inhibition by cobimetinib, in combination with cis-retinoic acid, represents an effective strategy for antitumor activity in refractory NB. In addition, the distinct alterations seen in cell signaling pathways provide means for effective biological correlative studies in future treatment protocols using MEK inhibition.

05 - Investigating the potential role of microRNA-21 and miR-31 in organotropic metastasis of lung cancer

Arvind K. Singla[1], Michela Garofalo[2], Gerard J Nuovo[2], Frank R. Jirik[1]
[1]Department of Biochemistry and Molecular Biology, The McCaig Institute for Bone and Joint Health, University of Calgary, Calgary, Alberta, Canada T2N 4Z6. [2]The Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA

Abstract: Lung cancer is the leading cause of cancer related deaths, leading to an estimated one million deaths per year. Even with intensive treatment schedules, the 5-year survival rate remains dismal, primarily due to local invasion and metastases. An incomplete understanding of the molecular and cellular mechanisms underlying metastasis has hindered the identification of effective therapies to treat this complication. MicroRNAs negatively regulate gene expression, and their dysregulation has been implicated in tumor initiation, progression and metastasis. We studied candidate microRNAs that might be involved in promoting metastatic progression. To generate metastases, NCI-H1299 cells ('Parental') were introduced into the arterial circulation of athymic (*nu/nu*), beige (NIH-III) mice. These cells metastasized to various sites, including adrenal gland, bone, and ovary. Cells derived from metastatic lesions were harvested, expanded *in vitro*, and characterized with respect to cytokine/chemokine and MMP secretion. We found significant heterogeneity among parental and organ specific sublines for the production of key cytokines/chemokine's and MMPs; which act by autocrine or paracrine mechanisms to sustain growth, induce angiogenesis, and to evade immune surveillance, thereby plausibly facilitating the growth of metastases. We also studied microRNA expression and expression of key microRNA target genes. Interestingly, a relationship between overexpression of oncogenic microRNAs (oncomirs), miR-21, and miR-31, and down-regulation of their respective tumor suppressive genes, Pdcd4 and Lats2, respectively, was observed. To validate the preclinical results in human tumors, expression of both miR-21/pdcd4 and miR-31/lats2 were examined in 120 human lung cancer tissues by in situ hybridization and immunohistochemical analysis. This novel *in vivo* lung cancer metastasis model and organ specific sublines provides a useful system for preclinical evaluation of novel microRNA or drug-based therapeutics specifically directed at the treatment of advanced stage lung cancer.

Acknowledgements: Canadian Cancer Society Research Institute and Alberta Cancer Foundation.

06 - Macrophage repolarization through STING activation: exploring local and abscopal effects on tumor growth

Oksana Yavorska, Charlene Downey, Frank Jirik
Department of Biochemistry and Molecular Biology, McCaig Institute for Bone and Joint Health

Mammalian cells have evolved multiple sensors to pathogen-associated molecular patterns, including the ability to respond to free dsDNA within the cytosole. One of the primary indirect DNA sensors, the stimulator of interferon genes (STING) is activated by cyclic dinucleotides generated by the DNA-responsive cGAS enzyme, and then stimulates various downstream effectors, including TBK1 (tank binding kinase-1), IRF3 (interferon regulatory factor-3), and STAT6 (signal transducer and activation of transcription-6). The latter induce type 1 interferons and NFκB-associated pro-inflammatory mediators. Recently, it was found that the tumor vascular disrupting agent DMXAA (5,6-dimethylxanthenone-4-acetic acid) was a STING ligand, suggesting that STING was a key target in tumor biology. Our lab recently published data showing that both DMXAA and 2'3'-cGAMP (the endogenous STING agonist generated by cGAS) was able to repolarize tumor-associated M2-like macrophages towards the inflammatory M1 macrophage subtype both *in vitro* and *in vivo*, suggesting that STING-induced macrophage repolarization was key to the anti-tumor effects of DMXAA. To investigate this further, we carried out a pilot study to examine the effects of intra-tumoral injection on DMXAA and 2'3-cGAMP on subcutaneous 344SQ-EGFP-Luc2 tumors. The 344SQ cell line was derived from a spontaneously forming NSCLC metastasis in mice harboring $p53^{R172H/g+}$ and K-$ras^{LA1/+}$ transgenes and transfected with an EGFP-Luc reporter (for *in vivo* bioluminescence imaging of tumors grown in immune-competent syngeneic hosts). Intra-tumoral injection of DMXAA or 2'3-cGAMP did not produce significant vascular disruption as compared to the classical intra-peritoneal route of administration, and in future we will determine whether injections of higher doses will lead to tumor necrosis as well as abscopal anti-tumor effects in this syngeneic model.

07 - Physical activity barriers and preferences among head and neck cancer survivors: participant experiences from the ENHANCE trial

Colleen Jackson[1], Lauren Capozzi PhD candidate, MD student[1], Harold Lau MD, FRCPC(C)[3,4], William Bridel PhD[1], S. Nicole Culos-Reed PhD[1,2,3]
[1]Faculty of Kinesiology, University of Calgary, Calgary, Canada [2]Department of Oncology, Division of Psychosocial Oncology, University of Calgary, Calgary, Canada [3]Alberta Health Services—Cancer Care, Tom Baker Cancer Centre, Psychosocial Resources, Calgary, Canada [4]Department of Oncology, Alberta Health Services, Calgary, Canada

Background: Head and neck cancer (HNC) is the sixth most common cancer worldwide, accounting for 3-5% of cancer diagnoses each year (ACS, 2014). Although there is a growing population of survivors due to advancements in treatment, many have decreased quality of life stemming from treatment-related side effects (Pulte et al., 2010). Among HNC survivors, participation in physical activity (PA) is associated with decreased fatigue, improved functional well-being and overall quality of life (QOL) (Rogers et al., 2006; Capozzi et al., 2014a; Capozzi et al., 2014b). Despite the numerous benefits, only 8.5% of a sample of HNC survivors were found to be meeting the PA guidelines, and over half were sedentary at 18.6±50.9 months post diagnosis (Rogers et al., 2006). These findings indicate the need to increase PA adoption and adherence with the goal of tailoring PA programs to meet the needs of HNC survivors, thereby positively impacting adoption and adherence. There has been a growing body of research investigating the PA preferences and barriers to address adoption and adherence issues among other cancer groups; however, only one study has been conducted investigating each of these topics among HNC survivors (Rogers et al., 2006;Rogers et al., 2009). Additionally none to date have qualitatively explored the factors that might enhance PA adoption and adherence over the cancer trajectory.
Purpose: The primary aims of this work are to determine; i) PA barriers experienced by HNC cancer survivors during and after their participation in the ENHANCE trial, an exercise intervention delivered during and after treatment; and ii) PA preferences of HNC survivors who have recently completed treatment (Capozzi et al., 2012). Secondary aims include examining how these PA barriers change throughout the course of participation in the ENHANCE trial.
Methodology: This mixed-methods approach will recruit HNC survivors who have participated in the ENHANCE trial. Participants will complete a self-report survey and a subset of participants who participated in the program at different intervals will be invited to participate in a semi-structured interview. Questions will include demographic information, PA levels, PA barriers and preferences, and QOL (FACT-AN).
Significance: This study will facilitate the further development of tailored PA programs for HNC survivors and provide an understanding of barriers and preferences that may facilitate PA program adoption and adherence. This may ultimately increase numbers of HNC survivors who engage in regular PA, thereby enhancing their quality of life.

Funding Support: Canadian Institute of Health Research CGS-M Grant. The ENHANCE trial is funded by Alberta Cancer Foundation and Joe's Team funding.

08 - The Role of Polynucleotide Kinase Phosphatase in Non-Homologous End Joining

Cortt Piett and Susan P. Lees-Miller. Department of Biochemistry and Molecular Biology, Southern Alberta Cancer Research Institute, Robson DNA Science Center, University of Calgary, AB

Ionizing radiation (IR) therapy is the predominant form of treatment for a wide variety of cancers, as IR induces numerous variations of genomic lesions which can reduce cell proliferation and growth. The most cytotoxic of these DNA lesions is the double strand break (DSB). A DSB occurs when single stranded breaks (SSBs) occur in close proximity to one another and may result in chromosomal separation. Importantly, IR induced strand breaks frequently contain non-ligatible 5' hydroxyl and/or 3' phosphate groups. Human polynucleotide kinase/phosphatase (PNKP) exhibits 5' DNA kinase and 3' DNA phosphatase activities, making it ideal for converting "dirty" DSB ends to compatible ends prior to ligation. The major pathway for the repair of IR induced DSBs in human cells is non-homologous end joining (NHEJ). PNKP interacts with the NHEJ scaffolding protein XRCC4 in a phosphorylation dependent manner (Koch et al, EMBO J, 2004), suggesting a mechanism by which PNKP is recruited to IR-induced DSBs, however, its precise role in NHEJ remains enigmatic. The current work examines the interaction between PNKP and XRCC4 and the DSB repair kinetics of PNKP deficient cells. PNKP interacts with XRCC4 in an IR dose-dependent manner, and the interaction with XRCC4 is affected by the inhibition of DNA-PKcs. DSBs can be visualized by staining cells for γH2AX, a molecular marker for DSB repair, and these foci are counted using immunofluorescence microscopy techniques. γH2AX foci scoring (in an isogenic background) has demonstrated that PNKP deficient cells exhibit a late DSB repair defect at times past 6 hours in asynchronously growing cells populations, and G1 phase cells exhibit a later DSB repair defect at times past 12 hours compared to the parental cell line. The stable re-introduction of PNKP into the knockdown cell line also rescues the observed late DSB repair defects. Lastly, live cell imaging is currently being used to evaluate PNKP recruitment/retention kinetics at localized laser induced DNA damage tracks in the presence and absence of inhibitors.

09 - Identification and characterisation of a novel phosphorylation site in the catalytic cleft of the DNA damage response and mitotic kinase, DNA dependent protein kinase catalytic subunit (DNA-PKcs)

Edward Bartlett, Pauline Douglas, Ruiqiong Ye, Shujuan Fang, Susan Lees-Miller
Robson DNA Science Centre, University of Calgary

Cancer progression is supported by genomic instability in tumour cells, which can be caused by DNA damage from both endogenous and exogenous sources. Our cells possess mechanisms to correct a myriad of DNA lesions, including the repair of DNA double strand breaks (DSBs). The primary pathway in higher eukaryotic cells for the repair of DSBs is non homologous end-joining (NHEJ), of which the DNA dependent protein kinase catalytic subunit (DNA-PKcs) is a core factor. DNA-PKcs is known to phosphorylate itself and other repair factors during the process of DSB repair. Another cause of genomic instability is aberrant cell division, or the misregulation of mitosis. Proper coordination of mitosis is achieved by an intricate network of phosphorylation (and dephosphorylation) events, with a number of key protein kinases known to orchestrate the process. Recently, we have shown that DNA-PKcs is also active during normal mitosis, and loss of the protein or its kinase functions results in aberrant mitosis. Here we demonstrate a novel phosphorylation event, phospho-serine 4026, in the catalytic cleft of DNA-PKcs, and observe its occurrence during both DNA repair and mitosis. The kinase domain of DNA-PKcs has been shown to adopt a highly similar structural conformation to that of mammalian target of rapamycin (mTOR) kinase by x-ray crystallographic studies. The high resolution of the mTOR structure allows us to postulate on the likely location of serine 4026 in DNA-PKcs, and to speculate on its role in regulation of kinase activity.

Funding from Eyes High and CIHR.

10 - Functional Tissue Imaging Unit. Protein Expression Analysis Using Multiplex Quantitative Immunofluorescence

Translational Laboratories, Tom Baker Cancer Centre, Calgary, Alberta.

Immunohistochemistry has been used for decades as a means to determine protein expression and localization in normal and diseased tissue. Its use as a screening tool has been limited, however, by the low throughput and procedural complexity. Here we present a workflow which allows for concurrent immunostaining, data acquisition and analysis of up to four protein targets in several hundred tissue samples. The procedure hinges on the combination of many samples into a tissue microarray (TMA), whereupon hundreds of samples can be stained concurrently on a single slide. Automated fluorescent immunostaining and whole-slide scanning reduce procedural variability, permitting single-cell or tissue-scale study of protein expression and relocalization. We demonstrate a protocol for TMA production, staining, and data analysis of four markers (including a nuclear marker) in formalin-fixed, paraffin-embedded (FFPE) human normal and cancerous tissue. We further demonstrate the use of image analysis software to quantify biomarker expression, and associate these data with various clinical indicators such as progression-free survival or overall survival.

11 - Regulation of epithelia-mesenchymal transition by the SUMO pathway: Implication for cancer cell invasion and metastasis

Ayan Chanda, Shorafidinkhuja Dadakhujaev, Amrita Singh Chandhoke, Lili Deng, Shirin Bonni
Department of Biochemistry and Molecular Biology and Southern Alberta Cancer Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

Tumor cell invasiveness and metastasis are the causes of cancer related-morbidity and mortality. Epithelial-mesenchymal transition (EMT) is a key cellular process that allows cancer cells to escape local environment and invade secondary sites leading to metastatic disease. The cytokine Transforming Growth Factor beta (TGFβ) induces EMT and promotes the invasive growth in a variety of cancers including breast cancer bone metastasis. Understanding the mechanisms that regulate TGFβ-induced EMT and invasive tumor cell growth should add valuable insights into how cancer metastasis can be controlled. We have shown that the PIAS1, a member of the SUMO post-translational modification pathway, regulates TGFβ-induced EMT and breast cancer invasion and metastasis. We have also found that TGFβ stimulation reduces the protein abundance of PIAS1. Studies are ongoing to determine if PIAS1 can be as used a therapeutic target in cancer. Our studies have significant implications in cancer therapy.

12- Exercise Improves Satisfaction with Life and Physical Function for Brain Cancer Survivors

Gregory Levin [1], Daphne Tsoi [2], Kenneth Greenwood [3], Robert Newton [3]
[1] University of Calgary, Calgary [2] St John of God Hospital, Subiaco, Australia [3] Edith Cowan University, Australia

High grade brain tumours are unpredictable and incurable with 5-year survival rates of 20%. The poor prognosis combined with intensive treatment results in high levels of distress and reductions in quality of life and physical functioning. Since curative treatments are usually not possible, the inclusion of complementary therapies to optimize quality of life while living with brain cancer should be considered. Exercise therapy has been shown to be beneficial in other cancer populations, but no evidence is available for brain cancer survivors. Therefore we report results from two preliminary case studies.

Two female participants 'A' (61y, 164.7cm, 59.5kg) 5-months post diagnosis of *glioblastoma multiforme* and 'B' (58y, 163cm, 66.4kg) 62-months post diagnosis of *oligodendroglioma* both undertook a 12-week supervised exercise program consisting of two 1-hour resistance and aerobic exercise sessions per week, and additional self-managed aerobic sessions. Assessments for maximal strength, aerobic capacity, psychological distress and self-efficacy were performed prior to and after the conclusion of the intervention.

Exercise was well tolerated and both participants successfully completed all 24 exercise sessions and additional self-managed sessions ('A'=44; 'B'=35) with no adverse effects. All outcome measures displayed positive responses relating to reduced morbidity (Table 1). Both participants exhibited similar psychological changes whereas greater physical improvements were obtained by 'A' despite undergoing regular chemotherapy treatments.

Table 1. Case study outcomes for two brain cancer survivors undertaking exercise

	Participant 'A'		Participant 'B'	
	Baseline	Week 12	Baseline	Week 12
Maximal strength	62 kg	92 kg	94 kg	110 kg
400m Walk test	250 s	231 s	259 s	248 s
Psychological Distress	24 / 42	13 / 42	23 / 42	14 / 42
Satisfaction with Life Scales	7 / 35	17 / 35	11/35	29 / 35
Self-efficacy	110 / 170	156 / 170	115 / 170	149 / 170

To our knowledge, these two case studies are the first to examine exercise as a treatment for patients with malignant brain tumours. The findings suggest that despite the difficulties associated with brain cancer treatment and survivorship, exercise is safe and beneficial and should be considered in the overall management of patients with brain cancer.

13 - The NuA4 complex promotes Translesion Synthesis (TLS) – mediated DNA damage tolerance

Margaret Renaud-Young, David C. Lloyd, Kate Chatfield-Reed, Iain George, Gordon Chua and Jennifer Cobb

Chromatin remodeling complexes have been implicated in the DDT pathways, and here we identify the NuA4 complex, which is a histone acetyltransferase, to have a potential role on the Translesion Synthesis (TLS) branch of DDT. Genetic analyses in *Saccharomyces cerevisiae* showed synergistic sensitivity to MMS when NuA4 alleles, *esa1*-L254P and *yng2*Δ, were combined with the *error-free* bypass mutant *ubc13*Δ. The loss of viability was less pronounced when NuA4 complex mutants were disrupted in combination with *error-prone*/ TLS factors, such as *rev3*Δ suggesting an epistatic relationship between NuA4 and *error-prone* bypass. Consistent with cellular viability measurements, replication profiles after exposure to MMS indicated that small regions of unreplicated DNA or damage were present to a greater extent in *esa1*-L254P/ubc13Δ mutants compared to *esa1*-L254P/rev3Δ and beyond the completion of bulk replication. Indeed, the critical role of NuA4 in *error-prone* bypass is functional after S phase. Underscoring this observation, when Yng2 expression is restricted specifically to G2/M of the cell cycle, viability and TLS-dependent mutagenesis rates were restored. Lastly, disruption of *HTZ1*, which is a target of NuA4, also resulted in mutagenic rates of reversion on level with *esa1*-L254P and *yng2*Δ mutants, indicating that the histone variant H2A.Z indeed functions *in vivo* on the TLS branch of DDT.

14 - Investigating the *In Vivo* Consequences Of Salicylate Co-Administration On The Efficacy Of Topoisomerase II Poison-Based Chemotherapy

Gina M.A. Crovetto[1,2], Jason T. Bau[1,2], Qiao Shi[1], Don G. Morris[1], and Ebba U. Kurz[1,2]
[1]Southern Alberta Cancer Research Institute and
[2]Department of Physiology & Pharmacology, University of Calgary

Human DNA topoisomerase IIα (topo II) is an essential nuclear enzyme involved in the untangling of DNA during DNA replication, transcription and mitosis. Topo II manages DNA tangles and supercoils by transiently binding and cleaving one DNA duplex to allow the subsequent the passage of a second DNA duplex through the opening. Several of the most widely used anti-cancer chemotherapeutics are topo II poisons, which exploit the enzyme's ability to induce transient DNA double-stranded breaks (DSBs) by trapping topo II in a covalent complex with DNA. This results in the accumulation of cytotoxic DNA DSBs. We previously determined that salicylate, the primary metabolite of aspirin, catalytically inhibits topo II in MCF-7 human breast cancer cells (Bau & Kurz, Biochem. Pharm. 2011). Furthermore, it was demonstrated that a brief pre-treatment with salicylate decreases the cytotoxicity of doxorubicin and etoposide, two widely used topo II poisons. Aspirin ranks among the world's most widely consumed pharmaceuticals and daily dosing for the prevention of secondary major occlusive vascular events is commonplace. The goal of our work is to determine the *in vivo* consequences of salicylate on the efficacy of topo II poison-based chemotherapy using a murine xenograft model of breast cancer. We first characterized the *in cyto* effect of salicylate co-administration on the DNA damage signaling and cytotoxicity of doxorubicin and etoposide in the human breast cancer cell lines MCF-7 and MDA-MB-231, and the murine breast cancer cell line EMT-6. We observed that co-administration of salicylate and the topo II poison doxorubicin attenuated the doxorubicin-induced autophosphorylation of ATM on serine 1981 and the ATM-dependent phosphorylation of p53, Chk2, and SMC1 in MCF-7 and MDA-MB-231 cells. We similarly observed an attenuation of doxorubicin and etoposide cytotoxicity in MCF-7, MDA-MB-231 and EMT-6 cells. Building on these results, we have initiated an animal-based study using a murine xenograft model carrying luciferase-bearing MDA-MB-231 tumours to investigate the impact of salicylate co-administration on the efficacy of topo II poison-based chemotherapy *in vivo*. The results of these experiments will provide insight that may inform future clinical practice concerning salicylate co-administration in women undergoing chemotherapy with topo II-targeting drugs for the treatment of breast cancer.

FUNDING:
Canadian Breast Cancer Foundation (to E.U.K)

15 – A novel distribution-based imputation method to optimize biomarker selection for metabolomics experiments

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Problems in metabolomic biomarker selection commonly arise because mass spectrometry instruments have limits of detection and finite measurement ranges . That is, for each metabolite, there is a floor value, a ceiling value, and an interval where precise measurement data cannot be determined. As a result, in many samples, when metabolites are below the level of detection, "zero" values are assigned or values are dropped altogether, limiting or possibly distorting the contribution of those metabolites to the metabolomic model. Those metabolites may still provide an important contribution to the metabolomic model, if the bias and reduced variability of the reduced metabolite distributions are corrected. In this work, a distribution-based imputation method is proposed to recover the missing data. Using a dataset from patients with colorectal cancer, the accuracy of biomarker selection based on the imputed data were evaluated, using area under the ROC curve obtained from partial least square discriminant analyses. We compared several other imputation methods to the proposed approach. Performance metrics were based on simulated data and real metabolomic data. The potential benefits of this research will be described, including the correct identification of important metabolites.

Key words: limit of detection, metabolomic biomarker selection, distribution-based imputation method, partial least square discriminant analysis

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16 – I*n vitro* growth inhibition and target modulation in pediatric leukemia cells with p16 deletion

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The development and use of novel therapeutics are important for the treatment of paediatric leukemia due to the increased incidence of relapse or chemoresistance to initial treatment. Paediatric leukemia with the $p16^{INK4a}$ mutation is especially susceptible to refractory disease. This study investigates the activity of a library of relevant pharmaceutical pipeline drugs to identify effective novel treatments through the use of *in vitro* cytotoxicity assays and combination studies. Novel therapeutic drug screening using 150 inhibitors determined 6 therapeutics with high activity against $p16^{INK4a}$ deleted cells as defined by low half maximal inhibitory values (IC_{50}) ranging from 0.2-1.0 *n*M. Drug combination studies used an effective cyclin dependent kinase inhibitor (CDK) PD0332991 along with 4 conventional chemotherapeutics and 2 novel therapeutics. Synergistic activity was observed with both novel therapeutics and 4 of the 4 conventional chemotherapeutics. Additive effects were observed in 2 of the chemotherapeutics. Using Decitabine, a DNA methyltransferase inhibitor, $p16^{INK4a}$ homozygous deleted cells were exposed to the therapeutic 18 hours prior to incubation with PD0332991. Results indicated that cells exposed to Decitabine prior to PD0332991 have two fold increased cytotoxicity compared to DMSO controls. Decitabine and 5'-Azacytadine are typically utilized in relapse patients and the potential combination with PD0332991 in $p16^{INK4a}$-deleted patients may provide increased recovery compared to single treatment regimens.

17 - Human colon cancer cells exhibit dual modes of cell death when treated with different concentrations of cisplatin

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Cancer cells that are treated with genotoxic agents can respond by repairing damaged DNA or initiating cell death through pathways such as apoptosis or checkpoint adaptation (entry into mitosis with damaged DNA) and we are using HT-29 human colon cancer cells treated with cisplatin to investigate further cell death responses. In cell viability assays we found that both 30 and 100 µM cisplatin were cytotoxic. However, light microscopy indicated that cells treated with these different concentrations were undergoing different modes of cell death, with 30 µM treated cells entering mitosis prior to death. Time-lapse video microscopy confirmed this observation, indicating that over 120 h 81% of 30 µM treated cells entered mitosis in comparison to 7% of 100 µM treated cells. Using immunofluorescence microscopy 93% of cells treated with 30 µM for 72 h, 93% of cells treated with 100 µM for 24 h and 94% of cells treated with 100 µM for 48 h were positive for γH2AX staining, a marker for DNA damage. Combining information from the above data we chose three groups of cells to study the different modes of cell death observed; 72 h 30 µM cells and 24 and 48 h 100 µM cells. Strikingly, in the 30 µM group, 95% of cells were positive for cyclin B and 8% were positive for the mitotic marker phospho-histone H3. By contrast, in the 24 h 100 µM group, 14% of cells were positive for cyclin B and 1% were positive for phospho-histone H3 while in the 48 h 100 µM group 7% of cells were positive for cyclin B and 2% were positive for phospho-histone H3. Western blotting showed that cells treated with 30 µM cisplatin were undergoing checkpoint adaptation; cells were positive for cyclin B, had low levels of phosphorylated-Y15 Cdk1 and had dephosphorylated Chk1 at S345. Examination of proteins that function in apoptosis revealed that 48 h 100 µM cells had low levels of the anti-apoptotic protein Mcl-1 and were positive for cleaved PARP, cleaved caspase 3 and cleaved caspase 9, whereas 24 h 100 µM and 72 h 30 µM treated cells contained Mcl-1 and did not contain cleaved PARP, cleaved caspase 3 or cleaved caspase 9. The change in pathways from checkpoint adaptation at 30 µM cisplatin to apoptosis at 100 µM cisplatin reflects the change from a pharmacologically relevant concentration (30 µM) to a suprapharmacological concentration (100 µM). We therefore predict that increasing our understanding of the relationship between checkpoint adaptation and apoptosis in treated cancer cells may help to identify pathways that could be used to improve current cancer treatments.

This project has been made possible through grants from Alberta Innovates Technology Futures and the University of Lethbridge Interdisciplinary Research Fund.

18 - Establishment and Drug Sensitivity, Gene Expression and Epigenetic Characterization of a Pediatric Leukemia Cell Line (POETIC1) with Primary Resistance to Decitabine

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Epigenetic alterations leading to the silencing of key tumor suppressor genes by promoter hypermethylation have been implicated in the pathogenesis of a number of malignancies, including leukemia. Currently, the prototypical DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine (decitabine) has been studied in a number of diverse protocols as an anti-leukemic agent. However, the pattern of non-responsiveness to decitabine appears to be complex and multifactorial with some patients showing primary resistance whereas others develop resistance following initial responsiveness. To further understand the molecular mechanisms that define growth regulatory networks in pediatric acute myeloid leukemia (AML), we established a unique cell line (POETIC1) from primary *in vitro* decitabine resistant blasts isolated from a child with refractory AML. These cells were then expanded and subcloned to generate stable lines that were then subjected to detailed molecular, epigenomic and drug sensitivity profiling. Transcriptome profiling of POETIC1 identified significantly up-regulated pathways including those involved in DNA repair, cell cycle regulation, oxidative phosphorylation and down-regulated genes affecting apoptosis and cell differentiation. Overall, our findings identified alterations in key cell cycle and metabolic pathways in leukemia cells with primary resistance to decitabine. Next, in vitro drug sensitivity assays were carried out against a comprehensive panel of pharmaceutical pipeline agents (n=142) with activity against known signaling growth regulatory pathways in leukemia to facilitate the identification of druggable targets in decitabine refractory cells in future therapeutics. The differential epigenomic and drug sensitivity signatures seen between POETIC1 cells and initial primary leukemic blasts provide important information regarding the generation of aggressive subclones to produce treatment resistance in children with leukemia. The POETIC1 cell line, is the first of its kind and provides a critical experimental tool to investigate the role of epigenetic alterations in leukemogenesis as well as the molecular and physiological mechanisms that define primary resistance to methyltransferase inhibitors. This cell line is expected to help in the identification of novel therapeutic agents for refractory disease in future clinical studies.

19 - The Role of Prolactin in the Cellular Response to DNA Damaging Agents

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High serum levels of the peptide hormone prolactin are associated with increased breast cancer risk and poor prognosis. Prolactin is also involved in breast cancer resistance to different chemotherapeutics. We identified that one isoform of the heat shock protein-90 (HSP90), Hsp90alpha, is a prolactin-Janus kinase-2 (Jak2)-signal transducer and activator of transcription-5 (Stat5) regulated gene in breast cancer cells. The overall goal of this project is to investigate potential pathways involved in prolactin induced resistance to DNA damaging agents with the hypothesis that the cross-talk between the prolactin pathway and the DNA damage response is important in the mechanism. In our studies prolactin increased the viability of breast cancer cells to DNA damaging chemotherapeutics over a period of 48 hours, and Hsp90 inhibitors, 17AAG and BIIB021, abrogated the effect of prolactin, indicating the mechanism of enhanced viability involves the master cancer chaperone Hsp90. The stability of Jak2 and both the total ataxia-telangiectasia mutated protein (ATM) and phospho-ATM appear to be dependent on functional Hsp90. Inhibition of Jak2 and ATM with specific inhibitors abrogated prolactin enhanced viability, suggesting their role in prolactin pathway. Interestingly, in long-term *in vivo* xenograft studies using breast cancer cells generated to express autocrine prolactin, autocrine prolactin increased the tumor latency of the DNA damaged cells in SCID mice compared to untreated or PRL or doxorubicin alone. We hypothesize that this is in part due to the cross-talk of the prolactin and DNA damage response pathways that may be affecting the tumor microenvironment.

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20 - Multi-modal metabolomics analysis of cachexia in head and neck cancer patients

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Cancer cachexia accounts for 20-30% of all cancer mortality, affecting more than 80% of cancer patients in the final weeks of life. Despite the high prevalence of cachexia, the underlying biological mechanism(s) is not fully understood. There is also a need for biomarkers that can inform clinicians of the development and status of cachexia as well as cachexia treatment efficacy. The aim of the present study is to investigate the metabolic profile of cancer cachexia in head and neck (HN) cancer patients, with a focus on the impact of a targeted exercise and nutrition intervention on the serum metabolome. Newly diagnosed HN cancer patients (N=60) were randomized into two groups undergoing either an immediate or delayed lifestyle intervention consisting of physical activity and nutritional support, as per the protocol by Capozzi *et al.*[1] developed to retard and prevent loss of muscle mass. All participants underwent cancer therapy (radiation or radiation and chemotherapy). Blood samples collected at baseline and at 3 months post enrollment will be used for the metabolomics experiments, applying the two complementary approaches, proton nuclear magnetic resonance spectroscopy (^{1}H NMR) and gas chromatography mass spectrometry (GC-MS). At month 3 the participants in the delayed group have not yet initiated intervention and will hence serve as a control group. Using univariate and multivariate statistics, several comparisons can be made, including comparing the (immediate) treatment group to the control (delayed treatment) group at 3 months to investigate the impact of the intervention. We will also compare treatment responders to non-responders, with an aim of identifying potential baseline metabolites that can be used to predict response. Finally, serum metabolomics analysis will be performed in the context of cytokine profiling in order to provide a more comprehensive coverage of possible biomarkers. In closing, serum metabolomics using multiple time points and intra-person comparisons may be able to provide insight into the underlying biological mechanisms of cancer cachexia and the response to the intervention. Previous efforts to delay or reverse cachexia have been largely unsuccessful. For HN cancer patients, this might partly be due to the late start of traditional cachexia treatment (i.e., nutrition intervention), occurring at best at time of symptom manifestation. In this regard, the immediate treatment in this study may prove to be more successful.

Reference: [1]Capozzi LC, Lau H, Reimer RA, McNeely M, Giese-Davis J, Culos-Reed SN: Exercise and nutrition for head and neck cancer patients: a patient oriented, clinic-supported randomized controlled trial. *BMC Cancer* 2012, 12.

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21 – Validation of a commonly used radiation treatment planning software for determining prognostic body composition metrics

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Background: There is a need for more robust prognostic and predictive factors in oncology. Sarcopenia (skeletal muscle depletion) is associated with increased morbidity and mortality in patients with colorectal, renal and stage III melanoma patients. Body composition analysis has been used in clinical and epidemiological studies to estimate presence of sarcopenia. However, body composition analysis is not used routinely in clinical oncology practice given the limited availability of software used for this purpose. SliceOmatic (Tomovision, Montreal, Canada) is the gold standard in computer software used to determine sarcopenia using CT imaging data. The importance of this study lies in the potential of more widespread use of sarcopenia, a novel prognostic factor, using Eclipse (Varian, Palo Alto, CA), a widely available radiation treatment planning software.
Objective: This study aims to assess whether Eclipse represents a valid tool for measuring body metrics when directly compared to sliceOmatic.
Materials and Methods: CT data from the radiation treatment planning scans of 45 patients (34 males, 11 females) with gastric or distal esophageal cancer treated with radical surgery followed by adjuvant chemoradiation between 2006 and 2009 were used for analysis. Mean BMI was 23.7 kg/m2 (range = 17.6 - 34.7 kg/m2). Body composition parameters were measured on a single transverse CT slice at the level of L3, both software programs were applied using the identical CT dataset for each patient. Body composition metrics included estimates of a total skeletal muscle (SMA), visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and intermuscular adipose tissue (IMAT) areas. Contours were derived automatically using Hounsfield unit parameters, then manually edited as required. The lumbar skeletal muscle index (SMA normalized for stature) and prevalence of sarcopenia using both programs were calculated. The Bland-Altman method was used to evaluate agreement between the two programs.
Results: Estimates of lumbar skeletal muscle area were highly correlated (R^2=98%) between Eclipse and sliceOmatic. The Bland-Altman method results demonstrated a mean difference of 3.54 cm2 with the 95% limits of agreement of -7.65 cm2 and 14.73 cm2. The average inter-method difference at the group level was 2.6% (95% CI 1.36%, 3.86%). Eclipse resulted in similar, but significantly lower, mean estimates of SMA compared to sliceOmatic (137.2 ± 36.4 cm2 vs 140.8 ± 36.5 cm2, $p < 0.01$), as well as mean lumbar skeletal muscle index (45.3 ± 8.9 cm2/m2 vs 46.4 ± 8.9 cm2/m2, $p < 0.01$). Prevalence of sarcopenia was 74% as determined by sliceOmatic vs 77% as determined by Eclipse (92% agreement, kappa = 0.79). Results were comparable between the programs for SAT but not for IMAT or VAT.
Discussion/Conclusion: Eclipse represents a valid tool for the accurate assessment of body composition measures used to determine sarcopenia. Although similar, results from Eclipse should not be used interchangeably or directly compared against measurements from sliceOmatic. Eclipse is not able to reliably measure visceral fat or intermuscular adipose tissue areas. Future studies evaluating sarcopenia as a prognostic and predictive factor in oncology patients using Eclipse software are ongoing.
Founding support: no funding required for this project

22 - Elucidating the role of Artemis through the cell cycle

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Artemis is a critical protein for the process of V(D)J recombination and also plays an important role in the repair of a subset of DSBs. Artemis appears to play roles in both NHEJ and HR repair, and is known to interact with DNA-PKcs, the MRN complex, DNA Lig IV, and PTIP. We sought to gain insight into the seemingly differential role Artemis plays in the NHEJ and HR repair pathways through the cell cycle. We observed that Artemis is highly phosphorylated in a mitosis dependent manner. Recent research has recorded that a number of DDR proteins are phosphorylated in a PLK1 and CDK1 dependent manner in mitosis to delay the DDR in mitosis. We propose that this mitotic phosphorylation of Artemis leads to the delayed activity of the Artemis protein in mitosis and that this mitotic phosphorylation will lend insight into the role that Artemis plays in both NHEJ and HR.

23 - Novel Role of Syntenin in Promoting Cell Growth and Invasion in Prostate Cancer through Modulating Snail Protein Level

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Introduction and Background: Syntenin is a scaffolding-PDZ domain-containing protein that is reported to be associated with the progression of human melanoma and other tumors growth and metastasis. However the role of Syntenin in prostate cancer (PCA) has not been previously explored. Recently, we identified Syntenin among a group of 10-gene signature with prognostic implication to PCA patients, in predicting biochemical recurrence and lethal disease. In this study, we sought to further investigate the possible role of Syntenin in PCA and identify the signaling pathways modulating this role. Herein, we examined the expression and function of Syntenin in prostate cancer cell lines and tumor samples.
Methods: Immunohistochemistry and Western blots were used to determine the protein expression of Syntenin in PCA patients' samples and various PCA cell lines. Knock-down of Syntenin with two different siRNA sequences in androgen dependent LNCaP and androgen independent C4-2 prostate cancer cell lines, was used to assess the corresponding biological changes in those cell lines. MTS cell proliferation, Flow Cytometry Cell Cycle Proliferation and cell invasion assays were used to assess the biological effects of Syntenin manipulations.
Results: Knockdown of Syntenin using siRNA resulted in significant decreases in cell proliferation growth of both androgen dependent and independent cell lines ($p= 0.002$ and 0.0063) respectively. However, it significantly reduced the cell invasion only in the androgen independent cell line C4-2 ($p=0.0017$), with no significant effect on the invasiveness of the androgen dependent LNCaP cell line ($p >0.05$). Those biological effects were correlated with significant changes of protein levels of cell cycle regulatory proteins (Cyclin D3 and p21) as well as downregulation of Snail as one of the crucial regulators of Epithelial Mesenchymal Transition. Syntenin expression in PCA patients' samples using immunohistochemistry was associated with PCA progression. Studies assessing the prognostic value of Syntenin in PCA are ongoing utilizing high throughput tissue microarray of 550 men with PCA.
Conclusion: Our findings demonstrate that Syntenin plays a significant biological role in PCA tumorigenicity, and reveal for the first time Snail as a novel downstream modulator of Syntenin signaling. Furthermore, our results suggest that Syntenin may contribute differently to the tumorigenesis of androgen dependent vs. androgen independent tumors. Syntenin warrants further investigation as a candidate molecular marker of prostate cancer metastasis and a potential therapeutic target.
Funding Support: Prostate Cancer Research Foundation, USA

24 - Testing A Panel Of Oncolytic Rhabdoviruses In Combination With Sunitinib (SU11248) In A Triple Negative Metastatic Breast Cancer Model

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Oncolytic rhabdoviruses (ORV) are viruses that preferentially infect cancer cells with defective interferon (IFN) signaling. In addition to directly killing tumour cells, ORVs can also engage the innate and adaptive host immune system to mount an anti-tumor immune response. However, in an immunocompetent host, the type I IFN in the non-cancerous tissues is still intact and therefore infecting the tumor with ORV is challenging when the virus is administered systemically. As a result the anti-tumor immune response may not be potent enough to clear the primary tumor. Sunitinib (Su) is an FDA-approved small molecule anti-angiogenic therapy that has also been shown to attenuate type I IFN response and reduce the number of immunosuppressive myeloid derived suppressor cells (MDSCs) in tumour bearing animals. Therefore, we hypothesized that sunitinib would improve ORV productivity in tumors and also enhance CD8+ T cell-mediated anti-tumor immunity, which would improve overall survival. Our preliminary experiments demonstrate that combining Su (40mg/kg) with ORV (VSVΔM51, Maraba-MG1) in an immunocompetent spontaneously metastasizing triple negative breast cancer model, 4T1, led to improved survival compared to Su or ORV monotherapies ($p<0.0001$). As predicted, Su increased viral infectivity in the tumor and reduced splenic MDSCs by >50%. Experiments measuring the activity of anti-tumor CD8+ T-cells and the effect of the co-therapy on metastatic lesions from the lung and the liver of 4T1 tumor bearing animals are currently underway.

25 - Smc5/6 complex maintains heterochromatin at telomeres

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Smc5 and Smc6 are two members of the Structural Maintenance of Chromosomes (SMC) family of proteins and the components of the Smc5/6 complex. SMC proteins also constitute the core members of the cohesin and condensin complexes. Like cohesin and condensin, the Smc5/6 complex is conserved among eukaryotes and is essential for cell viability. In addition to the SMC proteins, the Smc5/6 complex has 6 Non-Smc-Elements (Nse 1-6) that are essential for viability and affect the functionality of the complex.
Nse3 in *S. cerevisiae* is the homolog of human MAGE-G1 (Melanoma-associated antigen-G1) and a physical interaction has previously been reported between Nse3 and the telomeric protein Rif2[1] .We confirm this and find that a temperature sensitive mutant of *NSE3*, *nse3*-1, does not interact with *RIF2 and is defective* in Smc5/6 localization at telomeres. The loss of Nse3-Rif2 interaction has functional significance as *nse3*-1 mutants show a loss of telomere clustering at the nuclear periphery and dispersion of Sir4 proteins, which form distinct foci and maintain telomere silencing in wild type cells. Consistent with these results, we find that telomere silencing and global gene expression is dramatically altered in *nse3*-1 mutants. The loss of telomere silencing in *nse3*-1 cells was not altered by the further disruption of *RIF2*, which is known to provide more RAP1-binding sites for SIR proteins, promoting silencing[2]. In fact, *nse3*-1/*rif2*□ double mutants result in an additive defect in silencing. In line with Sir4 delocalization and the loss of silencing, telomeric noncoding RNA (TERRA) expression levels were dramatically increased in the *nse3*-1/ *rif2*□ double mutants compared to WT and single mutant cells. Interestingly, TERRA accumulation in *nse3*-1/*rif2*□ cells was specific to X-only compared to XY'- sub-telomeres, which is consistent with a previous report showing SIR proteins specifically regulate TERRA expression at X-only sub-telomeres[3]. Despite high level of TERRA expression, *nse3*-1/ *rif2*□ double mutants have shorter telomere length compared to *rif2*□ cells. Est2 levels are reduced in both *nse3*-1/ *rif2*□□and *nse3*-1 suggesting short telomere length in these cells is likely telomerase dependent. In all, we report a newly identified role for the Smc5/6 complex in heterochromatin maintenance and transcriptional regulation.
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26 - Identifying Novel Regulators of Epithelial-Mesenchymal Transition: Implication for Cancer Invasion and Metastasis

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Epithelial-Mesenchymal Transition (EMT) is a cellular process whereby epithelial cells lose cell-cell attachment and apical-basal polarity, and gain a fibroblastic phenotype and increased motility. EMT is fundamental in development, e,g for tissue and organ morphogenesis, and contributes to pathophysiological conditions including fibrosis and cancer. For example, EMT is induced in cancer allowing cells to break away from the primary tumor mass and reach distant sites and form new tumors or metastases. The secreted protein transforming growth factor beta (TGFβ) is a potent inducer of EMT in development and pathophysiological conditions such as cancer. Unraveling upstream regulators of TGFβ-induced EMT, which is a focus of the current study, should add insight into our understanding of how tissue morphogenesis is controlled during development and disease conditions. TCF7L2 is a transcription factor that is highly expressed in epithelial tissues of mammary glands and plays roles in maintenance and differentiation of epithelial cells. Interestingly, we have found that TGFβ reduces the protein abundance of TCF7L2 in cells undergoing EMT suggesting that TCF7L2 may regulate EMT. The objectives of our study are to determine if TCF7L2 regulates EMT in epithelial cells, elucidate the mechanisms by TGFβ downregulates TCF7L2 protein abundance, and examine the effect of TCF7L2 on the invasive and metastatic potential of breast cancer cells. Here we present our progress thus far. Overall, our ongoing research should provide the foundation for the potential development of novel therapeutic and diagnostic tools for treating breast cancer.

27 - Chemotherapy induced changes in the metabolome of colorectal cancer cells

Shahil Amin, Farshad Farshidfar, Karen Kopciuk, Hans J. Vogel, Oliver F. Bathe

Background: Systemic therapy remains a therapeutic mainstay for most patients with solid tumors. However, antineoplastic drugs are quite toxic and a significant proportion of individuals do not benefit. Therefore, an assay that would aid in early identification of individuals who benefit from a particular drug would be useful to minimize patient exposure to ineffective agents. Clinical benefit (a "response") is associated with tumor cell death and/or inhibition of cell growth. We postulate that a response to systemic therapy is associated with characteristic changes in extracellular metabolites, perhaps as a result of cell death or changes in other cellular processes. Our objective was to identify metabolites which changed as a result of a response to various systemic agents in colorectal cell lines.
Methods: Colorectal cell lines (HCT-116, HT-29, Caco-2 and HCT-8) were exposed to various doses of brivanib, oxaliplatin and 5-fluorouracil for 72 hours. Supernatant was collected at baseline and at 72 hours for metabolomic analysis. A MTT assay was done to identify conditions where cell growth was inhibited. The supernatant was analyzed by gas chromatography-mass spectrometry (GC-MS). Metabolites that changed in relation to treatment and response were identified.
Results: Each agent caused dose-related changes in supernatant metabolites. The metabolites associated with cell growth inhibition were distinct and different from dose related metabolomic perturbations seen in cells that survived. For example, in HCT-116 cells treated with brivanib, 79 metabolites changed in a dose-dependent manner. Of those, 14 metabolites were uniquely associated with cells that survived and 30 metabolites were uniquely altered in association with response.
Conclusion: In colorectal cancer cells, antineoplastic drugs cause distinct changes in the metabolome, and some of these changes are specifically associated with response. The list of metabolites that alter in association with response in our *in vitro* experiments comprise candidate metabolites that will be assessed in clinical samples from patients with colorectal cancer who are treated with systemic therapy. Identification of response-related changes in the circulating metabolome may represent a novel means of detecting response to chemotherapy, which may aid in personalizing systemic therapy.

28 - Examining How Ribosomal Proteins Affect Growth and Body Size

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This project is the result of a Markin USRP in Health & Wellness studentship

Adequate amounts of nutrients are important for growth and development. Ingested nutrients are used for processes that meet the metabolic needs of the body allowing for growth and development. An important aspect of growth is ribosome biogenesis. The ribosome is the molecular machine that is responsible for protein synthesis, hence growth. However, what still remains unclear is the contribution of rRNA and ribosomal proteins to tissue and body growth in developing animals. The aim of this project was to study the role of certain ribosomal proteins in growth and development using *Drosophila*. *Drosophila* is a powerful genetic model system to study cell and organismal responses to growth cues. Over a 4-day period, Drosophila larvae can grow over 200 fold in mass in response to nutrient availability. For this project a class of *Drosophila* mutants known as Minutes were studied. These flies harbored mutations in different ribosomal proteins. These flies are lethal as homozygous mutants, however as heterozygotes with reduced levels of ribosome and protein synthesis, they have a characteristic slow rate of development. The experiments conducted showed that mutations in certain ribosomal proteins result in no change or an increase in overall body size of *Drosophila* pupae in comparison to control. The results showed a link to delayed development to the pupal stage, suggesting an explanation to the increased body size. These findings showed a striking contrast to the hypothesized decrease in overall growth from decreased ribosome synthesis, opening the door to numerous questions regarding the molecular mechanism of ribosomal proteins.

29 – Defining mechanisms of oncolytic virus delivery using intravital imaging

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Oncolytic virus (OV) therapy is an approach to treating cancer that employs live viruses to seek and destroy tumor cells while concomitantly eliciting an immune response toward the cancer. Despite the fact that efficient delivery of the OV to tumour cells is critical for its therapeutic effectiveness, the mechanisms by which OVs are delivered to cancer cells *in vivo* remain largely unknown. Intravital microscopy (IVM) is a technique that allows real-time visualization of intact blood vessels and tissues in living animals. Recent reports of single virus particle tracking *in vitro* prompted our hypothesis that unique insights into how OVs are delivered from the circulation to cancer cells within a solid tumor could be gained by monitoring OVs *in vivo* using IVM.
We have developed a protocol to efficiently label Vesicular stomatitis virus ($VSV^{\Delta M51}$) with fluorophores without affecting viral productivity, allowing us to visualize individual viral particles using resonance scanning, fluorescence IVM. Within minutes, labeled virus was observed attaching to the endothelium within the tumor vasculature in virus-naïve mice harboring CT-26 subcutaneous tumors. In contrast, when a second dose of $VSV^{\Delta M51}$ was administered 24-72 hrs after the first treatment, the virus bound preferentially to monocytes ($CD11b^{+}$ / $Ly6C^{+}$) that had been recruited to the tumor vasculature and not to the underlying endothelium.FACS analysis of blood collected 5 minutes after viral injection confirmed the interaction between VSV and monocytes. Intriguingly, $VSV^{\Delta M51}$-coated monocytes made frequent, stable contacts with a population of neutrophils ($CD11b^{low}$/ $Ly6G^{+}$) within the tumor vasculature. Behavioral analyses indicated that $CD11b^{+}$ cells in $VSV^{\Delta M51}$ pretreated (24 h) tumors are more stationary in nature, as opposed to the crawling and probing phenotype these cells demonstrate in virus naïve animals. This suggests an increased state of cellular activation. Identifying the role that monocytes play in the delivery of virus to the tumor may prompt their targeting to help improve the efficacy of OV therapy.

30 – Anti-Thymocyte Globulin (ATG) at clinically relevant concentration induces complement-dependent cytotoxicity of leukemic blasts

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Background: Allogeneic Hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for patients with hematologic malignancies, particularly leukemia. Graft-versus-host disease (GvHD) is one of the major obstacles for successful HSCT. Polyclonal antithymocyte globulin (ATG), included in the conditioning regimens to enhance engraftment has shown to reduce the risk of both aGvHD and cGvHD without increasing the likelihood of leukemia relapse, raising the question whether ATG induces anti-leukemic effect. The precise action mechanism of ATG is still undefined. In this study, we used Thymoglobulin which contains polyclonal rabbit IgG directed against the human thymocytes to study relevant mechanisms of ATG-mediated anti-leukemic effects, including complement-dependent cytotoxicity (CDC), and apoptosis (complement-independent cytotoxicity).
Methods: In this study we assessed complement-dependent and "apoptosis" or complement-independent cytotoxic activity of ATG at clinically relevant concentrations (1, 10, 25 and 50µg/ml) in peripheral blood samples and/or bone marrow samples from 11 patients newly diagnosed with acute leukemia. Cytotoxicity was determined by staining with 7-amino-actinomycin D (7-AAD), annexin V and flow cytometry. Leukemic blasts were defined as $CD45^{intermediate}$.
Results: ATG at clinically relevant concentration (25µg/ml) induced significant complement-dependent cytotoxicity (CDC) of leukemic blasts from all the 11 acute leukemia specimens. However, significant complement-independent cytotoxicity (CIC) "apoptosis" of leukemic blasts by ATG was detected only at a concentration of 50µg/ml. It was also observed that ATG induced complement-dependent cytotoxicity of leukemic blasts in a dose-dependent manner.
Conclusion: ATG at clinically relevant concentration appears to kill leukemic blasts via CDC but not via CIC. For ATG killing leukemic blasts by CIC "apoptosis", higher dose of ATG would need to be given to patients (dose resulting in serum concentration of 50 µg/ml). Our preliminary data suggests that ATG in presence of complement induces substantial anti-leukemic activity, providing a possible explanation for favorable clinical data on leukemia relapse after allogeneic HSCT.

31 - Next Generation Sequencing Validation of *PIK3CA* Mutational Status in Cervical Cancer Patients Treated with Radical Chemoradiotherapy

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Funding Support: The Terry Fox Research Institute, Society of Gynecologic Oncology of Canada, Alberta Cancer Foundation, Alberta Innovates Health Solutions

Background and Aims: We have previously shown poor overall survival (OS) in cervical cancer (CC) patients treated with radical chemoradiotherapy (CRT) harboring tumor *PIK3CA* mutation (McIntyre *et al*, Gynecol Oncol 2013) using Sanger-based sequencing. The aim of this study was to re-sequence CC specimens using next generation sequencing (NGS) to determine *PIK3CA* mutational status reproducibility, and to evaluate tumors for additional potentially clinically relevant mutations.

Methods: Pre-treatment biopsies from 82 patients with CC treated with radical CRT from a single institution were evaluated. Tumor DNA was tested for *PIK3CA* mutation status and additional mutations using the Ampliseq Cancer Hot Spot Panel (CHPv2) on the Ion PGM using a 316v2 chip. Bioinformatic analysis was performed using Torrent Suite Software version 4.02. Effect of tumor *PIK3CA* status via NGS on OS was determined.

Results: NGS detected 100% of *PIK3CA* mutations previously detected using Sanger sequencing, as well as two additional *PIK3CA* mutation positive cases not previously identified. *PIK3CA* mutational frequency was 26% (21/82 cases), with 86% (18/21) in squamous cell cancers. Estimate of *PIK3CA* mutation effect on OS increased with NGS evaluation: age-adjusted HR 2.8 (CI 1.3-6.1, p=0.009); vs. Sanger: age-adjusted HR 2.4 (95% CI 1.1 – 5.4, p=0.032). Other mutations were comparatively rare: FBXW7 (5/82, 6%), KRAS (4%), NRAS (2%) and PTEN (2%).

Conclusion: NGS reliably validates *PIK3CA* mutational status initially identified using standard Sanger sequencing, and increases estimate of mutation effect on OS. We were able to further elucidate the mutational spectrum of these tumors, adding to clinically relevant molecular knowledge.

32 - Ribosome synthesis in *Drosophila* muscle is required to maintain systemic insulin signaling and larval growth

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The conserved Target-of-Rapamycin (TOR) kinase signaling pathway links nutrient availability to cell, tissue and body growth in animals. One important growth-regulatory target of TOR signaling is ribosome biogenesis. Studies in yeast and mammalian cell culture have described how TOR controls ribosomal RNA (rRNA) synthesis via the RNA Polymerase I transcription factor Transcription Initiation Factor - IA (TIF-IA). However, the role of TOR-dependent ribosome synthesis to tissue and body growth in animals is less clear. Here we show in *Drosophila* larvae that ribosome synthesis in muscle is required non-autonomously to maintain normal body growth and development. We find that amino acid starvation and TOR inhibition lead to reduced TIF-IA levels and decreased rRNA synthesis in larval muscle. When we mimic this decrease in muscle ribosome synthesis using RNAi-mediated knockdown of TIF-IA, we observe delayed larval development and reduced body growth. This reduction in growth is caused by lowered systemic insulin signaling via two endocrine responses: reduced expression of *Drosophila* insulin-like peptides (dILPs) from the brain and increased expression of Imp-L2 - a secreted factor that binds and inhibits dILP activity - from muscle. Finally, we show that activation of TOR specifically in muscle can increase overall body size and this effect requires TIF-IA function. These data suggest that muscle ribosome synthesis functions as a nutrient-dependent checkpoint for overall body growth: Upon nutrient rich conditions, TOR is required to maintain levels of TIF-IA and ribosome synthesis to keep high levels of systemic insulin, but upon starvation, reduced muscle ribosome synthesis triggers an endocrine response that limits systemic insulin signaling to restrict growth and maintain homeostasis.

33 - Investigating the role of LSPI motif containing partners of protein phosphatase 2A in mitosis

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Reversible protein phosphorylation is the most common post-translational modification which controls most cellular processes. Mitosis is tightly regulated by the activities of protein kinases and protein phosphatases. One of the primary functions of mitosis is equal segregation of sister chromatids into two daughter cells. This process requires high fidelity and precision. PP2A-B56 is an important mitotic protein phosphatase which contributes to the fidelity of the process of sister chromatid segregation by dephosphorylating certain outer kinetochore proteins and thereby stabilizing attachments between sister chromatids and the microtubules of mitotic spindle. PP2A-B56 is recruited to the mitotic spindle by BubR1, a mitotic protein kinase. The minimal amino acid sequence on BubR1 required for binding PP2A-B56 has been identified to be KL*p*SPIIE or the 'LSPI' motif. Repoman, another important mitotic regulator, also binds to PP2A-B56 through the same motif. We hypothesize that LSPI is a general consensus motif for recruitment of PP2A-B56, particularly during mitosis, and that additional proteins utilize this recruitment sequence. We speculate that recruitment of PP2A-B56 is regulated by the phosphorylation status of this motif and this in turn plays an important role in controlling the progression of the cell cycle. Our preliminary results show that two proteins with putative LSPI motif - (1) Anaphase Promoting Complex 1 (APC1) and (2) Inhibitor of Apoptosis Stimulating Protein of p53 (iASPP), also interact with PP2A-B56 in a phosphorylation dependent and isoform dependent manner. Interestingly, both the proteins are functionally linked to regulation of the cell cycle. APC1 is the largest subunit of the APC/cyclosome complex which functions as an ubiquitin E3 ligase and controls exit from mitosis. iASPP is a highly evolutionarily conserved inhibitor of the tumor suppressor protein, p53 and is reported to be involved in cell cycle regulation. Further understanding of how interactions between the target proteins and PP2A-B56 affect mitotic progression or exit will provide valuable insight about the mechanisms of specific recruitment and mitotic functions of PP2A-B56.

34 - Use of novel anti-inflammatory agents to inhibit osteosarcoma lung metastasis

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Osteosarcoma is the most common cancer of the bone and predominantly occurs in the adolescent population. Despite increases in survival among osteosarcoma patients with localized disease, the survival rate for patients presenting with metastasis to the lung is still less than 30%, identifying a need for novel therapies that inhibit pulmonary metastasis. Our growing understanding of the complex relationship between inflammation and cancer has led to the investigation of anti-inflammatory therapeutic approaches for targeting cancer. Many cancers arise at sites of chronic inflammation as seen with the occurrence of colon carcinoma in patients with inflammatory bowel disease. In addition, the tumor microenvironment, largely composed of and orchestrated by inflammatory cells, including neutrophils, has been shown to contribute to cancer progression, especially at metastatic sites such as the lung, by facilitating cell proliferation, survival, and migration. To date, non-steroidal anti-inflammatory drugs (NSAIDs) have shown some promise as treatments for metastatic disease, but this work has been limited. In this study we assessed the effects of two novel anti-inflammatory agents, LT-peptide and GML, on osteosarcoma lung metastasis. LT-peptide is a lung/liver targeting (LT)-peptide identified to bind to the endothelium of the lung and liver and inhibit neutrophil recruitment during inflammation. GML (GM1-Targeted, Linoleate-Containing TLR2 Ligand), an engineered anti-inflammatory ligand, has also been shown to inhibit neutrophil recruitment in models of chronic inflammatory disease (rheumatoid arthritis and colitis). To investigate the inhibitory potential of LT-peptide and GML in osteosarcoma metastasis to the lungs, we established an *in vivo* osteosarcoma lung metastatic model. Briefly, 143B human osteosarcoma cells stably expressing luciferase were injected intravenously via the tail vein into CB17 SCID mice in the absence or presence of LT-peptide or GML. Phosphate buffered saline (PBS) and a peptide that binds the lung but does not inhibit neutrophil recruitment were used as controls. Metastatic burden in the animals was visualized weekly using bioluminescence imaging (Xenogen, IVIS 200) and validated post-sacrifice by histological analysis (anti-human nucleolin staining). The number of metastatic lesions was quantified for all lobes of the right and left lung in five non-sequential histological sections. Preliminary data suggest that both LT-peptide and GML have inhibitory effects on osteosarcoma lung metastasis. Optimization of the concentration, dosing schedule, and route of administration of these agents are ongoing. The findings from these preclinical studies may provide insight for the development of a novel therapeutic approach for targeting osteosarcoma metastasis.

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35 - INhibitor of Growth 3 (ING3) modulates Androgen Receptor activity in prostate cancer

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Introduction: Prostate Cancer is the most common cancer among men worldwide and it is very highly age-related, with incidence increasing dramatically from the 5th decade of life onwards. Androgen Receptor (AR) is known as the key player in the progression of Prostate Cancer. The INhibitors of Growth (INGs) are plant homoedomain (PHD) proteins that serve as epigenetic "readers" and target epigenetic "writers" that acetylate or deacetylate lysine residues on histone tails. They also serve as type two tumor suppressors, and are deregulated in numerous types of cancer. ING3 is the third member of this family of proteins, which is a stoichiometric member of TIP60 histone acetyltransferase (HAT) complex. TIP60 modulates and acetylates histones and other proteins such as p53 and AR and play an important role in their functions.
Hypothesis: Since TIP60 is known as Androgen Receptor (AR) coactivator, we hypothesize that ING3 may be involved in AR signaling pathway by virtue of being a member of TIP60 complex and therefore can be involved in the progression of prostate cancer.
Results: Our studies on prostate cancer cell lines showed that ING3 was expressed more in AR positive cell lines, C4-2 and LNCaP and its protein levels can be increased by addition of androgen analog. Our studies on prostate cancer tissue samples indicated that ING3 is positively associated with AR and that higher levels of ING3 is correlated with poorer outcome in these patients. Knockdown of ING3 in LNCaP and C4-2 cells reduced the effects of androgen analog on downstream targets of AR. Furthermore, recruitment of AR on Androgen Response Elements (ARE) was reduced after transfection of ING3 siRNA, as tested by chromatin immunoprecipitation (ChIP) assay. Transactivation of AR was also tested by luciferase assay and results indicated that ING3 increased AR activity in a stoichiometric manner. Co-immunprecipitation studies suggest a possible interaction between AR and ING3, which is independent of DNA. Moreover, ING3 promoted the interaction of TIP60 and AR and therefore modulated the effects of TIP60 on AR activity.
Conclusion: Our results show that ING3 modulates AR activity by promoting TIP60 and AR interaction in prostate cancer cells. Protein levels of ING3 were correlated with patient survival, which further suggest ING3 as a novel prognostic marker in prostate cancer.

36 - A PDGF-AA Initiated Model of Glioblastoma with Predictable Onset

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Glioblastoma multiforme (GBM) is an almost uniformly fatal brain cancer that mostly occurs in older individuals and carries a five-year survival rate of less than 5%. Although the genetic and epigenetic landscape of GBM is now well annotated, its pathogenesis and critical therapeutic targets have remained elusive. By utilizing two well documented alterations of GBM, p53 loss of function, and activation of platelet derived growth factor receptor-alpha (PDGFR-α), we have created a novel *in vitro* system in which we can examine the cellular and molecular alterations that occur during the early stages of tumorigenesis. When cells from the subventricular zone (SVZ) of p53-/- mice are cultured in PDGF-AA, after 100 days in culture, they predictably transform, giving rise to a population of cells that proliferate continuously in the absence of exogenous PDGF-AA. Furthermore, when grafted into the brains of immunocompetent mice, they form cellular, invasive, GBM-like tumors. In contrast, p53 null cells grown in epidermal growth factor and fibroblast growth factor (EGF/FGF) proliferate rapidly *in vitro* but remain growth factor dependent and non-tumorigenic, until switched to PDGF-AA; 100 days later, they too become growth factor independent and tumorigenic. The marker profile of EGF/FGF grown cells, as well as the PDGF-AA transformed cells remains similar to OPCs throughout transformation, suggesting a similar progenitor cell which is transformed via culturing in PDGF-AA. Competitive inhibition of PDGF-AA binding to PDGFR-α inhibits cell proliferation pre but not post transformation. This simple, accessible model of human GBM might prove to be helpful for investigating the nature and sequence of early cancer causing events in neural precursor cells, inspiring new treatment strategies.

37 - Metabolic perturbations accompanying colorectal adenoma and colorectal cancer

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Introduction: Colorectal cancer (CRC)is the third most common cause of cancer death in North America.. The circulating metabolome is a function of tumor phenotype as well as the individual's metabolic response to the disease state. Our objective was to characterize the circulating metabolome in CRC patients. In addition, we sought to explore whether it was possible to detect a very early stage of disease (adenoma, a premalignant lesion) using the same approach.
Methods: In total, sera from 31 individuals with adenoma and 316 individuals with CRC, along with their age- and gender-matched controls, were analyzed using gas chromatography-mass spectrometry (GC-MS) in the Metabolomic Research Center (MRC). This provides a metabolomic profile for each case, which consists of measured metabolites' concentrations. We then evaluated the range of alterations by univariate and multivariate approaches. In addition to testing a discovery cohort, we performed an external validation in a separate cohort.
Results: The circulating metabolome in CRC patients is effectively perturbed compared to disease-free controls. This divergence is also visible in colorectal adenoma, but with a less pronounced magnitude of change. We also present performance measures in our external validation study and the clinical accuracy of the presented Adenoma- and CRC-specific metabolomic profiles. The signature for CRC could detect CRC with an accuracy of 89% in an independent validation cohort. Finally, we confirmed the presence of a stage-dependent progression of changes in the locoregional metabolomic profile, which is readily detectable by the metabolomic methodology.
Conclusion*:* Mass spectrometry-based metabolomic profiling is a compelling method for detecting phenotypic changes in the sera of CRC affected patients. This study confirms our prior findings on applicability of metabolomic phenotype analysis for detection of colorectal early lesions and advanced disease, and calls for prospective studies on the clinical applicability of screening and diagnostic tests, designed based on these validated signatures.

Keywords: Colorectal cancer, metabolomics, mass spectrometry, cancer biomarker

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38 - Hypoxia-mediated regulation of growth signaling in *Drosophila* larvae

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Hypoxia is a feature of most tumors. Resistance to hypoxia is associated with poor prognosis and resistance to therapy. Given its central roles in tumor progression and resistance to therapy, tumor hypoxia has been considered the best targets. However, despite an accumulation of information on tumor hypoxia, most information was obtained from cell-based experiments and there are still major questions to be addressed. Thus, we used *Drosophila* larvae to understand how whole animals regulate growth in response to hypoxia. We determined that hypoxic responses were triggered after 10 min treatment with 1% O_2. Growth signaling pathways such as Insulin/PI3K and TOR were not significantly affected by 10 min hypoxia. Interestingly, eIF2α, a eukaryotic translation initiation factor was highly phosphorylated in hypoxia, and phospho-eIF2α was rapidly dephosphorylated in normoxia (20% O2). Given that GCN2 and PERK kinases are involved in eIF2α phosphorylation in mammals and humans, we tested if these kinases are conserved in eIF2α phosphorylation in *Drosophila*. We observed that knockdown of either GCN2 or PERK decreased eIF2α phosphorylation induced by hypoxia, suggesting that GCN2 and PERK can directly or indirectly phosphorylate eIF2α in hypoxia. In addition, we also observed that expression of a mutant eIF2α (S50A) lacking the phosphorylation site reduced the phosphorylation of endogenous eIF2α. In the future, we will elucidate mechanisms of eIF2α phosphorylation in hypoxia and dephosphorylation of phospho-eIF2α in normoxia. We will also examine the biological significance of hypoxia in animal growth and development. Lastly, we will test the effects of GCN2 or PERK depletion on tolerance to hypoxia, possibly by inducing cell survival or blocking stem cell differentiation. The results of this study will provide insights on understanding why hypoxic tumors become more progressive and resistant to conventional therapies.

39 - Unraveling the role of Ku80 C-terminus region in non homologous end joining

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Non-homologous end joining (NHEJ) is the major DNA double strand (DSB) repair pathway in mammalian cells. The first step in NHEJ is recognition of the DSB by the Ku 70/80 heterodimer and the subsequent recruitment of the DNA-dependent protein kinase (DNA-PKcs), a serine/threonine kinase. DNA-Ku-DNA-PKcs together form the DNA-PK complex. This complex carries out DSB end alignment, activates Artemis endonuclease activity and finally recruits the XRCC4-ligaseIV complex which ligates the DNA ends. The aim of my project is to understand how Ku the heterodimer recruits DNA-PKcs to DSBs and activates its kinase activity.
The Ku heterodimer consists of 70 and 80 kDa subunits, and is conserved throughout evolution with some structural variations mainly at the C-terminal regions of both subunits. It has been reported that the extreme C-terminal 14 amino acids of Ku80 are required for DNA-PKcs recruitment and activation. However another study demonstrated that deletion of the Ku80 C-terminus does not abolish DNA-PKcs activation. In summary, there is considerable ambiguity regarding the role of Ku80 CTR in DNA-PKcs recruitment and activation and how interaction with Ku leads to activation of DNA-PKcs remains a critical question in the field. We hypothesised that Ku80 C-terminal deletion causes destabilization of the DNA-PK complex which leads to dissociation of the DNA-PK complex before it can perform its function, such as Artemis endonuclease activation.
Using clonogenic cell survival assays we confirmed that hamster cells expressing Ku80 with deletion of the CTR are radiosensitive and showed that these cells are sensitive to other genotoxic agents, namely etoposide, doxorubicin, neocarzinostatin. We generated Ku80 with C-terminus deletions (residues 1-718 and 1-569), cloned them into baculovirus vectors, and expressed and purified them from insect cells. *In vitro* reactions using purified DNA-PKcs showed that Ku heterodimer containing Ku80 residues 1-718 showed only slight defects in autophosphorylation whereas Ku heterodimer containing Ku80 residues 1-569 a.a) had major defects in DNA-PKcs autophosphorylation. Ku80 deletion mutants were also defective in phosphorylation of DNA-PK substrate proteins, XRCC4, XLF and PNKP. These findings support our hypothesis that the Ku80 CTR is important for NHEJ. Experiments to characterize the ability of these mutants to activate Artemis endonuclease activity are in progress.

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40 - The role of the CHD5 chromatin remodeller in the maintenance of Genomic Stability

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CHD5 is a known tumour suppressor, it was identified as part of a common chromosomal deletion frequently seen in Neuroblastoma, but has also been shown to be down-regulated or deleted in a wide range of cancers. Its loss is believed to act upstream to inactivate p53. CHD5 is a member of the CHD protein family of chromatin remodellers and is a member of the SNF2/RAD54 helicase family. CHD5 is classified as a member of CHD class II which also contains CHD3 and CHD4 both of which have established roles in genome stability.

Our interest lies in whether CHD5, a brain specific homologue of CHD3 and CHD4, has a role in maintaining genomic stability. We are working to ask whether CHD5's remodelling ability has an impact on its tumour suppressive functions. Particularly we are looking at the effects of specific deletions on the ability of cells to effectively repair IR induced damage.

We are also interested in the influence CHD5 is having on cell death and senescent pathways. It has an established impact on the p53 pathway, and we are working to see how this affects cellular decisions under genomic stress.

The importance of CHD5 is established by the fact that it is deleted or epigenetically silenced in a wide range of cancers, were its deletion correlates with poor prognosis. We suggest that the role of CHD5 as a tumour suppressor is dependent on either an impact on cell death/senescence decisions, chromatin remodelling for genome stability or a combination thereof.

41 - Radiologist Initiated Referral for Patients Suspected of Having Lung Cancer

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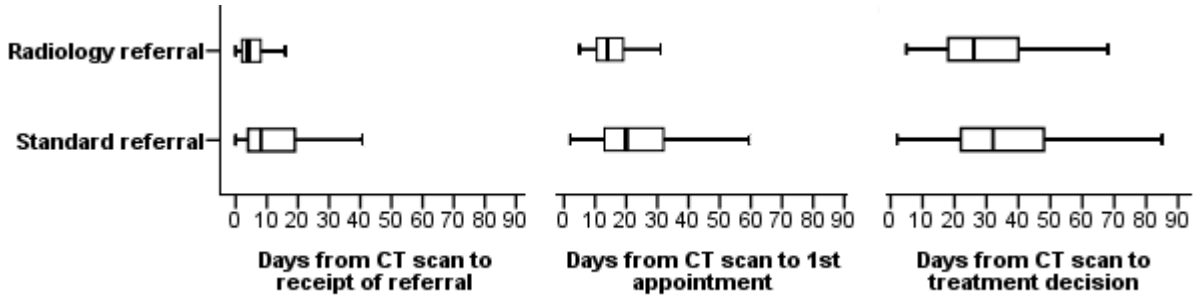
Background: The time interval between detection of a lesion suspicious for lung cancer on imaging and referral to specialty care is too long. Therefore, we aimed to evaluate a potentially faster referral process by having the radiologist trigger a referral to specialty care at the time of CT interpretation.

Methods: A prospective observational non-randomized study of two groups of patient referred to the Alberta Thoracic Program-South (ATOP-S) was carried out from December 2012 to September 2014 (total n=967): Group 1: Subjects who were referred to the ATOP-S clinic through a pilot program in which radiologist interpreting suspicious CT scans can directly initiate this referral at the time of interpretation. Group 2: Subjects who were referred to the ATOP-S clinic through another (non-radiologist) health care provider. The time interval between dates of 1st suspicious CT scan to receipt of referral (CT-R), 1st specialty appointment date (CT-A), and treatment decision (CT-D) were assessed. The differences in CT-R, CT-A, and CT-D time intervals between two groups were tested with the Mann- Whitney U-Test. P values < 0.05 (two-sided) were considered to be statistically significant.

Results: For 76 subjects in group 1, and for 840 subjects in group 2, CT-R, CT-A, and CT-D could be calculated. The median (75th-90th percentile) CT-R was 4 (8-13) days in group 1 and 8 (19-37) days in group 2. The median (75th-90th percentile) CT-A was 14 (19-26.3) days in group 1 and 20 (32-52.9) days in group 2. The median (75th-90th percentile) CT-D was 26 (40-63) days in group 1 and 32 (48-71) days in group 2. Subjects in group 1 significantly had a shorter CT-R, CT-A, and CT-D intervals compared to subjects in group 2 (P-values 0.000, 0.000, and 0.004, respectively).

Conclusion: A radiologist initiated referral program significantly reduced the interval between first CT scan suggestive of a lung malignancy to receipt of referral, 1st specialty appointment date, and treatment decision.



42 - The interplay of SUMO, Slx5/Slx8 and SMC5/6 complex ensures DNA double strand break relocation to the yeast nuclear pore

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In budding yeast, an irreparable DNA Double Strand Break (DSB) moves away from active sites of homologous recombination (HR) in the interior of the cell to the nuclear periphery where alternative methods of repair can occur. This phenomenon appears to be conserved in higher eukaryotes, and movement of DNA within the nucleus is an intriguing new type of regulation of DSB repair. The signal which triggers the relocation of breaks has not been identified, yet enzymes involved in the Small Ubiquitin-Like Modifier (SUMO) pathway have been suggested. We have analyzed the movement of an irreparable DSB in SUMO-ligase mutants *SIZ1*, *SIZ2*, and *MMS21*, and find that all three are deficient in break movement to the nuclear pore complex, but not to the nuclear envelope-bound protein Mps3. Furthermore, a SUMO mutant which is unable to form poly-SUMO chains is deficient in movement of a break to the nuclear periphery. All together, our data suggests that the accumulation of SUMO chains on one or more proteins is a signal that triggers break movement to the nuclear periphery where non-canonical repair can occur.

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43 - The molecular mechanism of CHD6 in the preservation of genome stability and cancer prevention

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CHD6 (Chromodomain, Helicase, DNA binding 6) is a class III CHD/Mi2 family ATP-dependent chromatin remodelling enzyme that, via interactions with the NFE2-related factor 2 (NRF2), is part of a protein complex that responds to oxidative stress – a key feature of IR exposure – and regulates the expression of many detoxifying enzymes following exposure to reactive oxygen species (ROS), thus maintaining cellular redox homeostasis. CHD6 has raised interest since several human ataxias have linkage map regions that encompass the CHD6 gene locus on chromosome 20q11.1-12. Further, catalytically-inactive CHD6 mutant mice exhibit motor coordination defects most consistent with a cerebellar neuron disorder (i.e. ataxia). Since ataxia is commonly seen in human syndromes lacking normal DNA strand break responses, this raises the possibility that CHD6 contributes to some aspect(s) of the DNA break response. It is, therefore, of interest to our understanding of human IR responses to investigate the role of CHD6 within the cellular response to DNA damage.

Preliminary data we have obtained implies that CHD6 protein expression increases substantially within one hour after exposure to IR and *tert*-butyl hydroperoxide (TBH), although how this increase occurs is not yet apparent. In addition, CHD6-GFP is recruited to UV-A laser DNA damage tracks as rapidly as one minute after induction, is absent by 10 minutes and is dependent upon the enzyme PARP. Further, CHD6 protein expression increases after treatment with proteasome inhibitor Bortezomib implying the expression of the enzyme is regulated by the proteasome. Based on evidence from the literature and my preliminary data, I hypothesize that CHD6 is involved in the signal transduction response following DNA damage and thus contributes to genomic stability and cancer prevention.

44 - Outcomes in stage I non-small cell lung cancer following the introduction of stereotactic body radiotherapy in Alberta– A population-based study

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Purpose: To evaluate determinants of outcomes for stage I Non-Small Cell Lung Cancer (NSCLC) patients following the introduction of stereotactic body radiation therapy (SBRT) in Alberta, Canada.
Methods: SBRT cases were linked to the provincial data base along with clinical, treatment and health services parameters for all cases of stage I NSCLC between 2005 and 2011. The pre-diagnosis Aggregation Clinical Risk Grouping score (ACRG3) was categorized into 4 categories: 10-19=1, 20-49=2, 50-69=3, 70-99=4 for risk analysis as a proxy for pre-treatment patient comorbidity. A logistic regression model was used to examine outcomes. Concordance statistic (c-statistic) and modeling were used to determine whether study variables predicted for overall survival (OS).
Results: 2146 patients were examined. Overall median age was 72 years. 43.0% of cases had a 1 year pre-treatment ACRG3 of 10-49. SBRT utilization rate increased annually and had an OS superior to conventional RT (median survival [MS] of 39.4 VS. 23.5 months, $P<0.001$) despite more patients having higher ACRG3 scores. Surgical patients were younger, had lower ACRG3 and achieved the longest MS at 69.6 months. Regression analysis indicated both surgical intervention ([HR]=0.23,95%CI=0.18-0.28) and SBRT (HR=0.33,95%CI=0.21-0.51) remained most strongly associated with OS. Patient's ACRG3 (HR=0.79, $P<0.001$) and age (HR=0.83, $P=0.03$) were also independent factors strongly associated with survival. The C-statistic study model result of 0.86 (95%CI: 0.81-0.90) indicates an impact of treatment selection factors on OS.
Conclusion: Stage I NSCLC patients treated with surgery have the best outcome. SBRT demonstrates improved OS compared to conventional radiotherapy for non-surgical candidates. In the absence of a randomised trial, treatment selection factors in Stage 1 NSCLC are strongly associated with OS.

45 - Identification and validation of potential driver genes in human prostate cancer using an integrative oncogenomic approach

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Prostate cancer (PCa) is the most commonly diagnosed cancer among Canadian men. Despite effective diagnosis for indolent tumors, prostate cancer represents the second leading cause of death in Canada with a projected 4000 deaths in 2015. Clinical findings such as Prostate-Specific Antigen (PSA) concentration, Gleason score and tumor invasiveness staging are used to predict the disease outcome. A considerable percentage of indolent prostate cancer cases can progress to aggressive forms which require immediate therapeutic intervention. Accurate prognostic biomarkers are critical to avoid over-diagnosis and over-treatment which associated with higher morbidity. Oncogene amplification and overexpression is a common genetic pathway driving prostate tumorogenesis and cancer metastasis. However, the full amplicon landscape in this heterogeneous disease has yet to be elucidated. Here, we utilized a combined biological and bioinformatics approach to characterize novel PCa genes with prognostic implications. We hypothesize that identification of amplified genes with oncogenic bioactivity will improve prognostication, and may identify potential therapeutic targets.
To identify novel amplicon that contains potential oncogenes, we utilized Oncomine database and queried The Cancer Genome Atlas (TCGA) PCa cohort for the top 10% of amplified and simultaneously overexpressed genes to characterize associated genes in PCa. Coincidentally, four of those genes, GARS, AQP1, CRHR2 and GGCT, mapped to a 55 kb region within chromosome 7q14. AQP1, encoding Aquaporin 1, and GARS, encoding glycyl-tRNA synthetase, have been associated with cancer but not PCa. To investigate this in PCa, we queried large, multicenter PCa cohort from Memorial Sloan Kettering Cancer Center (MSKCC). We found that GARS and GGCT are co-amplified and overexpressed in 8% in metastatic PCa, Furthermore, both genes showed significant association with patients' prognosis ($P=0.0003$, $P=0.005$), respectively. To identify the prognostic value of those four genes, we utilized Tyler Cohort using a model based on Cox Proportional hazard method. Our data shows that the over-expression of all four genes has the most significant impact on disease outcome with hazard ratio 2.3.
Based on our preliminary findings, we are evaluating the co-amplification of those genes in our PCa tissue microarrays which comprise of 1100 cases with detailed clinical outcome over 10 years. This is by conducting a FISH-based assay using custom design bacterial artificial chromosome (BAC) FISH probes flanking the area of amplification. We are quantifying the frequency of individual genes amplification and the co-amplification of the four genes as well. Expression of those genes is being assessed by immunohistochemistry and those with strong prognostic value will be further subjected to *in-vitro* studies using RNA interference (RNAi) gene knockdown technique to investigate their potential roles in cancer proliferation, migration and invasion.
Our study would enhance our understanding of the molecular genetics of prostate cancer and provide a new insight into the underlying mechanisms of disease progression.

Supported by Prostate Cancer Canada.

46 - Reversible phosphorylation of the RVSF motif in PP1 regulatory proteins controls PP1 docking during the cell cycle

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Background: Regulation of the cell division cycle is critical for the maintenance of genomic integrity at the cellular level. Reversible protein phosphorylation is a prevalent regulatory post-translational modification that is catalyzed by protein kinases and phosphatases in the cell. PP1 is a major serine/threonine phosphatase in eukaryotes responsible for one-third of all dephosphorylations and gains specificity through regulatory subunits that recruit PP1 to unique protein targets. The interactions between the regulatory subunits and the catalytic PP1 subunit occur via the well-characterized RVxF motif. Mapping of the phospho-proteome during the cell cycle shows increased net phosphorylation of proteins during mitosis including the RV(S/T)F motif from regulatory proteins of PP1. We speculate this reversible phosphorylation of the RV(S/T)F motif might play a general role in association/dissociation of PP1 from its regulatory proteins during the cell cycle and be crucial to maintain phosphorylation of PP1 targets during this event

Objectives:

- Determine if the phosphorylation within the RVSF motif of regulatory proteins controls PP1 binding.
- Elucidate the significance of the reversible phosphorylation of the RVSF motif during cell cycle, particularly in mitosis.
- Establish if this phosphorylation and reversible PP1 binding is a key regulator that drives the cell cycle.

Methods and Results: Our results from *in-vitro* PP1 overlays confirm preferential PP1-binding with dephosphorylated RV(S/T)F peptides over phosphorylated RVp(S/T)F peptides. Mitosis-specific phosphorylation of the RVSF motifs has been shown by immunofluorescence studies using a pan-RVSF phospho-antibody. This phosphorylation has also been confirmed by enrichment of phospho-RVSF containing proteins in mitotic immunoprecipitation experiments as identified by mass spectrometry.

Conclusions:

- PP1 preferentially binds to the dephosphorylated peptides *in-vitro*.
- Increased phosphorylation within the RVSF motif during mitosis as seen by cell imaging and western blots.
- The phosphorylation within the RVSF motif might be regulated by the mitotic kinase Aurora B.

Funding: Alberta Cancer Foundation and Canadian Breast Cancer Foundation.

47 - Ras-ERK signaling controls growth in imaginal discs and intestinal stem cells via synthesis of rRNA and tRNA

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Over the last two decades, genetic studies in *Drosophila* have identified many of the improtant cell signaling pathways and networks that control cell and tissue growth. One such pathway is the Ras-ERK pathway. Ras- ERK has been shown to promote cell growth and proliferation in many tissues throughout *Drosophila* development. Moreover, overactive Ras-ERK signaling is observed in many cancers and can drive tumorgenesis. An important challenge is to identify how the Ras-ERK pathway influences cellular metabolism to drive growth. Here we report on the control of both rRNA and tRNA synthesis as growth effectors of Ras-ERK signaling. We show that activation of ERK signaling pathway by overexpressing activated forms of EGFR, Ras or Raf in wing imaginal discs leads to an increase both rRNA and tRNA synthesis. Similarly, expression of oncogenic RasV12 in *Drosophila* S2 cells increases tRNA levels, while pharmacological inhibition of ERK signaling by the MEK inhibitor, U0126 leads to reduced tRNA synthesis. RNA polymerases I (Pol I) and III (Pol III) transcribe rRNA and tRNA respectively. We previously identified the Pol I and Pol III factors, TIF-IA and Brf, as regulators of cell and tissue growth in *Drosophila*. Here we show that knockdown of either TIF-IA or Brf blocks the effects of Ras-ERK signaling on growth and proliferation in larval wing imaginal discs, adult midgut progenitor cells and adult intestinal stem cells. We are investigating how Ras-ERK signaling regulates TIF-IA- and Brf-dependent transcription. Given the importance of both rRNA and tRNA synthesis in ribosome biogenesis and mRNA translation, our studies point to control of protein synthesis as an important effector of Ras-ERK signaling in regulating growth.

(Funded by AIHS post-doctoral fellowship and CIHR operating grant)

48 - Oncolytic virus immunotherapy: Evaluating the importance of oncolysis in heterologous prime-boost by utilizing CRISPR-Cas9 genome editing technology to knockout the LDLR gene family

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Introduction: A recent breakthrough in cancer therapy employs infectious oncolytic viruses (OVs) to induce immunotherapy by activating the host immunity to target cancerous cells. Viral genetic malleability is exploited to engineer OVs expressing tumor antigens, such as human dopachrome tautomerase (hDCT). In particular, a prime-boost strategy comprised of non-oncolytic Adenovirus-hDCT (Ad-hDCT) with oncolytic Maraba MG1-hDCT (MG1-hDCT) is effective for treating metastatic B16-F10 murine melanoma. An analogous heterologous prime-boost strategy is presently in Phase 1 human clinical trials, thus emphasizing the importance of understanding the precise mechanisms behind this therapy. Current OV ideology assumes virus induced lysis of cancerous cells, or oncolysis, by the booster vector MG1-hDCT is a necessary adjuvant for the induction of immunotherapy that is responsible for tumor regression and durable cures. Unfortunately, this theory lacks definitive evidence and some suggest the highly immunogenic viral oncolysis serves as a distraction. Therefore, this assumption based on fundamental OV ideology will be evaluated in this proposed project to determine whether oncolysis is beneficial, neutral, or harmful towards immunotherapeutic efficacy of the heterologous prime-boost strategy.
To evaluate the therapeutic role of oncolysis, there are two specific aims in this study:
Aim 1. Construction of a murine melanoma cell line (B16-F10) resistant to Maraba MG1 virus infection. The novel CRISPR-Cas9 genome editing tool will be harnessed to knockout the Low Density Lipoprotein Receptor (LDLR) gene family, the cellular receptors for Maraba virus infection thereby eliminating virus induced lysis of melanoma cells. The double nickase strategy utilizing the Cas9 D10A nickase mutant will be employed to initiate and direct homology directed repair (HDR) within the B16-F10 cell line. When applied for genomic editing, HDR demonstrates an exceptionally high level of control, thereby permitting selective knockout of the genes for LDLR related protein associated protein 1 and LDLR. The knockout of these two genes are expected to interfere with proper protein expression of the LDLR gene family thus conferring resistance to Maraba virus infection and subsequent virus induced lysis. Polymerase chain reaction, Western blot, and Maraba virus infectivity studies will be performed to confirm gene editing and resistance to Maraba virus infection.
Aim 2. Determine the role of OV-mediated cell lysis on the therapeutic efficacy of heterologous prime-boost therapy *in vivo*. B16-F10 tumor bearing mice will be created by intravenous injection of the wild-type or LDLR knockout melanoma cells constructed in Aim 1 into C57Bl/6 mice. After 5 days of tumor growth, mice will be first treated with Ad-hDCT (intramuscular) followed 10 days later by MG1-hDCT (intravenous). Animal survival will be monitored and compared with control treatments to evaluate the effect of oncolysis on the efficacy of the prime-boost strategy. Results to date will be presented.
Significance: The implications of this project are significant in influencing current and future designs of OVs and development of prime-boost immunotherapeutic strategies. In particular, with the recent entrance of heterologous prime-boost into human clinical trials, understanding whether oncolysis helps or hinders the therapy will be important for engineering improvements into the promising strategy.

Funding Support: Alliance for Gene Therapy, Alberta Children's Hospital Foundation

49 - PI3K/AKT/mTOR pathway as a potential therapeutic target in cervical cancer

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Cervical cancer remains a significant global health issue, despite the development of a cervical cancer vaccine. It is the third most common cancer in women with an estimated 500,000 new cases and 270,000 deaths annually. In Canada in 2014, it was estimated that 1450 women will be diagnosed with cervical cancer and 380 women will die from the disease. Earlier we have shown that mutation in exon 9 of PIK3CA in patients with early stage (IB/II) disease correlated strongly with poor survival. We hypothesize that the PIK3CA E545K mutation results in activation of the PI3K signaling pathway, resulting in poor treatment response and treatment with clinically relevant PIK3CA/mTOR inhibitors will inhibit PI3K signaling and thus resulting in better treatment response. In our studies we have characterized HeLa cells, SiHa cells (cervical cancer, HPV+, PIK3CA wt) and CaSki cells, Me180 cells (cervical cancer, HPV+, PIK3CA-E545K) with respect to viability, DNA damage signaling (in response to ionizing radiation IR and cisplatin). We will validate our findings in cervical cancer cell lines in which PIK3CA has been depleted stably by shRNA and shRNA resistant wt-PIK3CA and PIK3CA E545K. We will also determine whether PIK3CA/mTOR inhibitors inhibit proliferation and enhance survival after exposure to IR and cisplatin both in vitro (cell lines) and in mouse xenograft models. Our in-vitro and in-vivo results, may guide future clinical trials for use of P3K/mTOR inhibitors in cervical cancer patients with PIK3CA mutation.

50 - What controls breast cancer mediated osteoclastogenesis?

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Bone metastases occur in 65-80% of patients who are suffering from metastatic breast cancer. The current therapies are not effective enough and new bone metastases will still occur in 30-50% of patients. Thus, we need new therapies urgently. Details regarding the mechanisms of bone metastases are still not clear.
Breast cancer metastases of the bone are primarily osteolytic. An osteoclast is a type of bone cell that breaks down bone tissue (bone resorption). Osteoblasts are instrumental in controlling the amount of bone tissue: osteoblasts form bone, and also help produce osteoclasts that resorb bone. The growth of bone metastasis in breast cancer might be induced by osteoclast and osteoblast recruitment, which could enhance tumour growth resulting in the "vicious cycle" of bone metastases. In the vicious cycle, tumour cells secrete soluble factors that stimulate the osteoblast mediate specialization of osteoclasts. Mature osteoclasts then break down the bone and this releases growth factors that stimulate tumour growth. We have however, discovered an osteoblast-independent mechanism that is stimulated by the prolactin (PRL) treatment of breast cancer cells. Prolactin is a pleiotropic peptide hormone and cytokine that is secreted from the pituitary gland, as well as normal and cancerous breast cells. Experimental and epidemiologic data suggest that prolactin is associated with the occurrence of breast tumors in postmenopausal women.
Our previous research uncovered that PRL and the prolactin receptor (PRLR) play an important role in osteoclastogenesis (the development of osteoclasts). 1) There is a significant association between high expression of PRLR with a shorter time to bone metastasis in human breast cancer patients, indicating a patho-physiological relevance; 2) PRL induces secretion of soluble proteins, which encourages the formation of mature osteoclasts. The details of the mechanisms by which PRL and the PRLR affect bone metastasis or the metastatic environment are not clearly known. Thus, we hypothesize that PRL promotes bone metastasis and osteolysis by the regulation of breast cancer secreted proteins. The results showed that human PRL enhanced osteoclastogenesis in a dose-dependent manner through conditioned medium from breast cancer cell lines. PRL did not enhance the levels of macrophage colony-stimulating factor in breast cancer cells.. The hedgehog pathway inhibitor, cyclopamine, reduced human PRL dependent and independent ostoclastogenesis. A, hedgehog ligand might be one of the secreted proteins that enhanced by PRL, which encourages the formation of mature osteoclast.
We hope that advances in understanding the role of PRL and breast cancer secreted proteins will continue to yield new and exciting therapeutic targets and insight into cancer metastasis in bone.

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51 - Investigating the role of STAT3 signalling in the migration and invasion of brain tumour initiating cells.

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Glioblastoma multiforme (GBM) is the most common form of adult malignant brain tumour, with a post-treatment median survival of approximately 14 months. The disease initiation, highly aggressive nature of GBM and resistance to treatment and recurrence have been proposed to be partly due to a subpopulation of brain tumour initiating cells (BTICs) that exist within the tumour. BTICs may also contribute to the invasive phenotype of GBM. The Weiss lab has established a library of patient-derived BTICs. Signal transducer and activator of transcription 3 (STAT3) signalling is highly activated in GBMs. Our group has previously demonstrated that STAT3 is activated in BTICs and blocking its activity results in decreased proliferation in vitro and increased survival in mice orthotopically xenografted with BTIC lines. We hypothesized that STAT3 signalling might then also be involved in invasion processes of GBM. The aim of this study is to demonstrate that inhibition of STAT3 activation can effectively reduce the migration and invasion of BTIC cells. Using two drug inhibitors to block STAT3 function, we studied three invasion markers: ICAM1, MMP2 and SERPINE1, which are transcriptionally regulated by STAT3. Our results show that STAT3 inhibition in BTICs reduces expression of these markers across multiple BTIC lines, *in vitro*. These results add support to the argument that STAT3 is crucial to the GBM disease process, and should be considered as a relevant clinical target for GBM disease management.

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52 - Permanent Breast Seed Implant (PBSI): What is the Effect of Arm Position on Post-Implant Dosimetry?

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Permanent breast seed implant (PBSI) is a novel method for the treatment of early-stage breast cancer. It is a one-day, outpatient procedure that can be an option for some patients following breast-conserving surgery. Radioactive Pd-103 seeds are permanently implanted in and around the seroma, the fluid-filled cavity that previously contained the tumour, at planned locations using preloaded needles. The Tom Baker Cancer Centre in Calgary, Alberta began performing PBSI in November 2013, and has treated seventeen patients to date.
The planning for PBSI is done with the patient's ipsilateral arm raised; however, anatomical changes and variations are unavoidable as the patient resumes her daily activities, potentially resulting in significant deviations in implant geometry from the treatment plan as the radiation is delivered over the subsequent months. The purpose of this study is to quantify the impact of ipsilateral arm position on the geometry and dosimetry of the implant. Following implant, patients undergo computed tomography (CT) scans on day 0 (i.e., immediately after implant) and day 60. At each of these times, scans are taken in both the arm up position, recreating the position of the planning scan, and in the arm down position.
Postplans involve the comparison of pre- and post-implant CT scans for analysis of the dose delivered to the seroma. The seroma is contoured by the radiation oncologist on the pre-implant CT scan; it is therefore necessary to transfer this contour to the post-implant scan. Three different methods have been explored to accomplish this contour transfer. The first involves rigid registration, where the seroma is rigidly transferred from the pre-implant to the post-implant CT scan without accounting for anatomical deformations between scans. This method has inherent errors, as the seroma volume can be expected to change between scans, due to seroma resolution or edema, as well as changes due to arm position. The second method involves the re-contouring of the seroma volume onto the post-implant CT scan. The disadvantage of this method is the challenging visualization of the seroma due to seed artifacts. It can also be skewed by observer bias. The third, most promising method, uses deformable image registration. In this method, anatomical deformations between two CT scans are first determined, and structures are then manipulated according to those deformations.
Preliminary results indicate that the effect of arm position on dosimetric parameters is extremely dependent on the method of analysis used, as the change in seroma volume can be a driving factor in determining the dose delivered to target volumes. Results using rigid registration and seroma recontouring have suggested a hotter implant, while results from deformable registration have suggested a colder implant with the patient's ipsilateral arm down. These parameters require further investigation for the determination of the appropriate method of analysis. This will additionally allow for determination of dosimetric variations for nearby structures, such as the heart and lung, at the two different arm positions.

53 - Motion of Organs at Risk in High Dose Rate Gynecological Brachytherapy

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Introduction: High Dose Rate (HDR) brachytherapy is part of the treatment protocol for advanced cervical cancer. The planning process for Magnetic Resonance (MR) guided treatment requires both Computed Tomography (CT) and MR imaging. This combines the high soft tissue contrast of the MR images with the ability of the CT scan to visualize the applicator inserted inside the patient. However, multiple patient transfers increases the overall patient motion, which can lead to changes in the position and volume of the organs at risk (OAR) in the pelvic region, such as rectum and bladder.

The purpose of this study is to evaluate the effect of bladder preparation (often used to reduce bowel dose) on OAR motion during HDR brachytherapy of cervical cancer.

Methods: Nine cervical cancer patients were treated without introducing bladder filling. Six patients had their bladder contents emptied and re-filled to a specific volume (120 – 200 cc). The change in bladder volume and center of mass position between the MR and CT imaging sessions, as well as the concordance index of the contoured bladder on both image sets were calculated using the Eclipse treatment planning system.

Results: Preliminary statistics suggest that patients with bladder preparation had a significantly higher concordance index for contouring of the bladder (0.74 ± 0.06) compared to the patients with no bladder filling (0.59 ± 0.19). In addition, prepped bladders show a mean positional change of 4.2 mm (range: 1.6-8.5 mm), while a positional change of 5.3 mm (range: 1.5-15.4 mm) is observed in the absence of bladder filling. The rectum shows an overall positional change of 6.6 ± 4.6 mm over the whole patient population.

54 – Cell autonomous and cell non-autonomous roles of p75 neurotrophin receptor ($p75^{NTR}$) in glioma invasion

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Human glioblastoam is a heterogeneous tumor composed of tumor cells and a small population known as brain tumor initiating cells (BTICs) or glioblastoma stem-like cells. BTICs appear to drive tumor progression, underlie therapeutic resistance and have been highlighted as therapeutic targets for patients with malignant glioma. The ability of glioma cells to invade into the surrounding brain parenchyma is a major clinical issue rendering glioblastoma incurable by conventional therapies. In a previous study, we found that the p75 neurotrophin receptor ($p75^{NTR}$) significantly enhanced invasion and migration of genetically distinct glioma by a cell autonomous mechanism. In addition, $p75^{NTR}$ was frequently observed in a highly invasive population of cells from freshly resected patient specimens. Importantly, $p75^{NTR}$ was found to mediate glioma invasion by neurotrophin-dependent regulated intramembrane proteolysis (RIP). Blocking of $p75^{NTR}$ proteolysis by the generation of cleavage-resistance mutants, or treatment of animals bearing $p75^{NTR}$-postive intracranial tumors with γ-secretase inhibitors, significantly inhibited glioma invasion and prolonged survival. Using a large panel of patient-derived-BTICs we have investigated the role of $p75^{NTR}$ in the stem-like compartment. Here we investigate the biological effects of $p75^{NTR}$ down-regulation on glioma derived BTICs. Immunocytochemical studies western blot analysis reveal that $p75^{NTR}$ is variably expressed on BTICs and that treatment with γ-secretase inhibitors significantly decreases BTIC invasion in 3D cultures *in vitro*. Down-regulation of $p75^{NTR}$ using shRNA significantly decreases BTICs invasion, proliferation and self-renewal ability. Moreover, $p75^{NTR}$ was present on as a component of BTIC-derived extracellular vesicles (EVs) that are implicated in tumor cell invasion through a cell non-autonomous mechanism. We found that $p75^{NTR}$ containing EVs promote invasion of non-invasive glioma cells. The composition of $p75^{NTR}$ containing EVs and their roles in glioma invasion are currently been investigated.

55 - CRISPR-Cas9 mediated gene knockout in a murine breast cancer model

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Cancer is a heterogenous disease that is notoriously elusive and difficult to treat. Oncolytic rhabdovirus therapy (OVT) is considered a promising candidate for therapeutic treatments and has recently been shown to synergize with a class of drugs referred to as SMAC mimetic compounds. Treatment with SMC/OV co-therapy induces upregulation of pro-inflammatory cytokines including TNFα, which binds to its receptor protein TNF-R1 to elicit a cellular death pathway - an event referred to as bystander killing. The mediatory role of TNF-R1 in bystander killing was demonstrated in vitro. The bystander effect was abrogated in TNF-R1 gene knockdown cells. In vivo, however, we have found that the host's adaptive immune system is critical for tumor clearance after co-therapy. CD8 T-cell depletion in mice completely abolished the survival advantage elicited by SMC/OV co-therapy. To effectively transition SMC/OV co-therapy into the clinic, it is critically important to understand the molecular and cellular mechanisms involved in tumor clearance. We hypothesize that TNF-R1 mediated tumor cell death is a necessary yet insufficient first step in a multistep mechanism and that the recruitment of the host's apdaptive immune system is necessary to achieve tumor clearance. To test our hypothesis, we are generating TNF-R1 k/o EMT6 breast cancer cells using a novel gene editing technology known as CRISPR-Cas9. TNFR1-deficient cells are resistant to TNF-mediated cell death, and will therefore allow us to determine the role of bystander killing on co-therapeutic efficacy. Balb/c mice will be engrafted with WT, TNFR1 k/o or chimeric WT/TNFR1 k/o cells to establish breast tumours. 10 days later, mice will be treated with SMC and OV and monitored for tumor cell survival. We expect primary death of WT EMT6 cells via TNF mediated death pathways and subsequent secondary death of TNF-R1 k/o EMT6 cells via recruitment of the adaptive immune system. To date, I have generated EMT6 clones expressing dox-inducible Cas9, and am currently confirming gene editing. Undersanding how OV- SMAC mimetic co-treatment elicits complete tumour responses will enable us to predict the likely therapeutic efficacy on a particular patient group.

56 - Influence of telomere dynamics on disease progression and therapeutic response in bone marrow failure syndromes

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Background: Aplastic Anemia (AA) is a bone marrow failure disease where insufficient levels of hematopoietic cells are produced. In approximately 15-25% of patients, the AA evolves into a myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Studies have demonstrated an association between shortened chromosome ends, advanced AA and increased risk of developing blood cancers. Heterozygous mutations in the gene encoding the telomerase protein component hTERT, are seen in 15% of patients with AA, resulting in shortened telomeres.
Purpose: Determine how these telomerase mutations and shortened telomeres impact disease progression and response to therapeutics.
Methods: To understand the biochemical properties and cellular consequences of mutant hTERT expression we have generated expression constructs that correspond to two specific hTERT mutations found in patients with familial AA and progression to MDS or AML. Constructs were expressed in a leukemic cell line (THP-1), catalytic activity was measured using the telomeric repeat amplification protocol (TRAP) and cell cycle analysis was performed using a double thymidine block, followed by flow cytometry to measure DNA content. Cells were cultured for 72 hours in the presence of therapeutics and viability was measured by Alamar Blue.
Results: We have demonstrated that both hTERT mutants retain telomerase activity *in vitro* and we are investigating the consequences of their expression in leukemic cells lines. Preliminary data suggests that expression of A202T mutant hTERT protein in THP-1 cells, results in a delay of the G1/S transition, which may have profound implications during hematopoiesis, negatively impacting the development and differentiation of mature blood cells. In addition, THP-1 cells expressing mutant hTERT proteins are more resistant to treatment with specific chemotherapeutic agents that are commonly used to treat patients with AML. Additional assays will be performed to characterize biochemical properties of the mutations, and to examine the function in hematopoiesis.
Conclusion: Resistance to therapeutics suggests that protocols used in the treatment of leukemia might have differential efficacies depending on whether cells express wild-type or mutant hTERT. By defining the role of telomeres in hematological disorders, it may be possible to alter treatment strategies based on predicted outcomes from our investigations. Our studies will advance our understanding of bone marrow failure and AML disease progression in patients with hTERT mutations as well as lead to novel therapeutics for bone marrow failure syndromes.

Funding: AIHS[a], CIHR[a], CRS[b]

57 - An Investigation of Parent and Child Sleep Patterns on an In-Patient Oncology Unit

Gaya Narendran, Soeun Lee, Dr. Lianne Tomfohr and Dr. Fiona Schulte

Sleep is vital for innumerable processes in a developing child. Pediatric cancer patients and their caregivers often stay in hospital for extended periods of time and report disruptions in usual sleep patterns. The primary aim of this research is to study objective as well as subjective measures of sleep quality in pediatric cancer patients, as well as their parents, receiving care in the oncology ward at the Alberta Children's Hospital. The effects of sleep disruption were investigated as they relate to the fatigue and level of anxiety experienced by this population. Data obtained from this research will help to identify ways to decrease the effects of an important physiological consequence of current cancer care practices thus, results from this study will be used to develop guidelines for hospital practices (i.e. the frequency of routine blood draws) relevant to sleep interruption in order to enhance the quality of the experience of staying in the hospital. Hospitalized pediatric cancer patients experience significantly disrupted sleep experiences, compared to previously published data outlining the sleep experience of pediatric cancer patients who sleep in their home environment. Increased nocturnal awakenings translate into greater self-reported anxiety and mood disturbance the following day, in both patients as well as their parents. Importantly, our study focuses on the total family unit and aims to address both the physical and psychological aspects of wellness in children and their families who are impacted by pediatric cancer. Results: This study is still ongoing. Our preliminary findings indicate that 80% of pediatric oncology patients self-report symptoms of sleep disruption and/or fatigue based upon measures of Child Sleep Habits, Anxiety and Sleep Quality. Additionally, the PedsQL Multifatigue Scale indicated that all 8 children reported greater general, cognitive and sleep/rest fatigue in comparison to a control group of 163 children hospitalized for renal function observation.

58 - Precursor States of Brain Tumor Initiating Cell Lines Are Predictive of Survival in Xenografts and Associated With Glioblastoma Subtypes

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In glioblastoma multiforme (GBM), brain tumor initiating cells (BTICs) with cancer stem cell characteristics have been identified and proposed as primordial cells responsible for disease initiation, recurrence and therapeutic resistance. However, the extent to which individual, patient-derived BTIC lines reflect the heterogeneity of GBM remains poorly understood. Here, we applied a stem cell biology approach and compared self-renewal, marker expression, label retention and asymmetric cell division in 20 BTIC lines. Through cluster analysis, we identified two subgroups of BTIC lines with distinct precursor states, stem- or progenitor-like, predictive of survival after xenograft. Moreover, stem and progenitor transcriptome signatures were identified, which showed a strong association with the proneural and mesenchymal subtypes, respectively, in the TCGA cohort. This study proposes a new framework for the study and use of BTIC lines and provides novel precursor biology insights into GBM.

SACRI RESEARCH SYMPOSIUM

GENERAL INFORMATION

Registration: MacEwan Conference & Events Centre, MacEwan Hall Foyer
Programs will be available at registration

Venue: All talks will be held in MacEwan Hall A

Breaks and Meals:
Both morning and afternoon breaks will be held in MacEwan Hall A
Buffet lunch will be served in MacEwan Hall B

Poster Session:
The Poster Reception will be held in the MacEwan Hall Foyer

**Poster boards will be set up and available for poster display at 2:00pm*
Poster removal to take place after dinner prior to 8:30pm

Banquet Dinner: Will be held in MacEwan Hall B.
Meal ticket will be provided at registration to be presented at the dinner banquet to your server

Custom Plated Dinner Menu:

Fresh Baked Rolls with Creamery Butter

Plated Starter Salad
Butterleaf Lettuce Wedge Baby Stripe Beets, Julienne Radish, Roasted Yellow Pepper, Dill Feta Charred Lemon Vinaigrette

Plated Entrée – pre selected choice of:
6oz Tenderloin with Sour Cherry Dijon and Rosemary Demi-Glaze Rubbed with House Steak Spice, Creamed Spinach and Wild Mushroom Capote.
or
Miso Maple and Ginger Marinated Black Cod with a Citrus Beurre-Blanc
or
Lentil and Grilled Vegetable Stuffed Roast Tomatoes with a Roasted Pepper Coulis

Starch: Dauphinoise Potato

Vegetables: *Olive Oil Roasted Cauliflower, Garlic Brocolini, Mini Peppers*

Plated Dessert
White Chocolate Raspberry Cheesecake

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