Cancer Research Institute of Northern Alberta

RESEARCH DAY 2015

Saturday, November 14th
University of Alberta
CCIS 1-440

UNIVERSITY OF ALBERTA
CANCER RESEARCH INSTITUTE
OF NORTHERN ALBERTA (CRINA)

WELCOME



Dear CRINA Research Day Attendee,

Thank you for joining us at the second annual CRINA Research Day. Last year at our inaugural event we welcomed over 300 attendees and featured more than 150 posters from many departments and faculties across campus. We are happy to announce that many of those attendees signed up to be members of CRINA, forming the core of our cancer research community. One year later, we continue to build our cancer research community by hosting a cancer-themed Research Day yet again. This year, we have given trainees an opportunity to both organize the program and present their work orally to our cancer research community at the University of Alberta. Further, we are emphasizing multidisciplinary collaborations that are so important for studying the complexities of cancer, by featuring team oral presentations. We hope that you continue to explore what the University of Alberta has to offer in the cancer research sphere and grow your network of collaborators through future CRINA Research Days.

CRINA as an institute has a well-established reporting structure with operations committees and advisory boards. At our core, we continue to strengthen connections within our cancer research community by hosting events throughout the year such as seminars and symposia. Our leadership team is working on defining U of A cancer research strengths in terms of both, research brilliance and available equipment, with plans to build on this excellence to accelerate discovery and innovation. CRINA also represents the interests of its members as a unified voice on the provincial stage, working with AHS, AIHS and ACF. Our ultimate goal is to establish our Institute as a national leader in cancer research and patient care, wherein clinical outcomes are addressed with scientific inquiry and where research drives innovations in cancer prevention, treatment and survivorship.

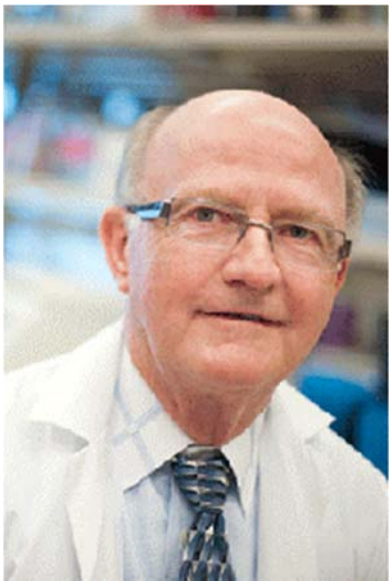
We hope you will enjoy today's program and find time to strengthen or make new collaborations with the CRINA community.

Sincerely,

Lynne-Marie Postovit | David Eisenstat | Catherine J Field

Dr. Lynne-Marie Postovit	Dr. David Eisenstat	Dr. Catherine Field
Interim co-director, Basic Research, Cancer Research Institute of Northern Alberta	Interim co-director, Applied Research, Cancer Research Institute of Northern Alberta	Interim co-director, Education, Cancer Research Institute of Northern Alberta

MESSAGE FROM THE VICE PRESIDENT, RESEARCH



An estimated 76,600 Canadians will lose their battles with cancer this year; more than 6,000 of them will be Albertans. To improve outcomes for patients and families with cancer, the University of Alberta has created the Cancer Research Institute of Northern Alberta (CRINA), one of the three multidisciplinary Translational Science Institutes (TSIs) dedicated to fostering collaboration between researchers to move the latest discoveries from the laboratory to the clinic.

This exciting new initiative has tremendous potential—CRINA will redefine the standard of cancer care in Canada by improving our understanding of cancer biology, discovering new therapies and biomarkers to diagnose patients with greater accuracy, and improving relapse rates and prevention.

I congratulate the life sciences faculties on this important interdisciplinary initiative.

Lorne Babiuk
Vice-president, Research
University of Alberta

PROGRAM

8:30 AM – 9:00 AM	**BREAKFAST**	CCIS PCL Lounge
9:00 AM – 9:05 AM	**WELCOME** David Evans, FoMD Vice-Dean Research	CCIS 1-440
9:05 AM – 9:55 AM	**ORAL PRESENTATIONS** Chair: Catherine Field	CCIS 1-440

- Gregory Cairncross (CRINA Leadership Invited Speaker)
  Alberta Cancer Research: Overview and Commentary
- David Kramer
  A proteomic investigation into the mechanism of chemotherapy-induced lymphoma tumour death
- Seyyed Kazemi-Bajestani
  Cancer fatigue progression strongly correlates with cancer cachexia development in advanced non-small cell lung cancer
- Zelei Yang
  Role of cytomegalovirus infection on breast tumor growth in mice

10:00 AM – 11:00 AM	**POSTER SESSION 1/SNACKS** ODD NUMBERED POSTERS	CCIS PCL Lounge
11:05 AM – 12:00 PM	**ORAL PRESENTATIONS** Chair: Helen Steed	CCIS 1-440

- Jack Tuszynski (2015 FoMD Tier 1 Excellence in Mentoring Award Winner)
  Challenges and rewards of HQP training in a multi-disciplinary cancer research environment
- Jihane Mriouah
  Fusogenic targeted liposomes as next-generation nanomedicine for prostate cancer
- Amanda Swan
  Mathematical modelling of brain tumour growth

- Hilmar Strickfaden and Christian Foerster

  Multicolor Electron Microscopy: Developing the next generation tool-kit for super-resolution microscopy in cell-biology

12:00 PM – 1:00 PM	**LUNCH/NETWORKING**	CCIS PCL Lounge
1:05 PM – 1:55 PM	**ORAL PRESENTATIONS** Chair: John Mackey	CCIS 1-440

- Chris McCabe (CRINA Leadership Invited Speaker)

  It takes a village to translate a technology
- Cameron Hough

  Biomedical applications of intense pulsed terahertz radiation
- Andre Isaac

  Droplet digital polymerase chain reaction for early detection of human papilloma virus – related head and neck cancer: a prospective validation study
- Matthew Benesch

  Autotaxin is an inflammatory mediator and therapeutic target in thyroid cancer

1:55 PM – 2:05 PM	**SHORT BREAK**	CCIS 1-440
2:05 PM – 3:00 PM	**ORAL PRESENTATIONS** Chair: David Eisenstat	CCIS 1-440

- Alisha Kadam

  EGFL7 inhibits cancer progression by suppressing angiogenesis through its Emilin-like domain
- Hina Vahidy

  Rituximab monotherapy as initial treatment for Post-Transplant Lymphoproliferative Disorder (PTLD)
- Mustafa Babadagli

  Integer linear programming optimization models for practical implementation in interstitial low-dose rate prostate brachytherapy
- Hashem Etayash

  Nanomechanical sensing of circulating tumor cells

- Rachel Fung and Brittany Umer

  Combining vaccinia virus with guided radiation therapy for the treatment of breast cancer and glioma

3:00 PM – 4:00 PM	**POSTER SESSION 2/SNACKS & BAR** EVEN NUMBERED POSTERS	CCIS PCL Lounge
4:05 PM – 4:50 PM	**ORAL PRESENTATIONS** Chair: Charles Holmes	CCIS 1-440

- Luc Berthiaume (Trainee Invited Speaker)

  Taking advantage of epigenetically induced essentiality at the NMT1 locus to develop a precision medicine approach to treat B cell lymphomas with a N-myristoyltransferase inhibitor

- Anais Medina and Susan Richter

  Design concept of smart viral nanoparticles for targeted molecular imaging and treatment of prostate cancer

- Sarah Purcell

  Accuracy of resting energy expenditure prediction equations in patients with advanced lung or colorectal cancer

4:50 PM – 4:55 PM	**CLOSING REMARKS**	CCIS 1-440
5:00 PM – 6:30 PM	**AWARDS, RECEPTION & CASH BAR**	CCIS PCL Lounge

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Speaker Abstracts

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Oral Presentations

1. A Proteomic Investigation into the Mechanism of Chemotherapy-Induced Lymphoma Tumour Death

David Kramer
Biochemistry

The EL4 mouse lymphoma tumour model has been extensively used in the field of medical physics as a model for developing methods to image tumour death. As the chemotherapeutic regimen to induce tumour death utilizes cyclophosphamide and etoposide - an alkylating agent and topoisomerase inhibitor, respectively - the form of tumour death has typically been assumed to be apoptosis. However, during the 2 day drug administration, tumours typically experience a 70 percent decrease in mass; this observation is indicative of tumour lysis syndrome, a condition characterized by the rapid and often violent eradication of cancerous tissue which can be detrimental to patient health. To assess whether the mechanism of tumour death in this model system is indeed apoptosis, EL4 tumours grown in C57/BL6 mice were treated with a combination of cyclophosphamide (2mg) and etoposide (1.5mg), or DMSO control for a period of 2 days. Tumours were excised, homogenized, and analyzed by mass spectrometry. Extracted ion chromatograms for each identified protein were used to determine relative protein abundance per tumour between the chemotherapy-treated and control tumour samples. Using this technique, 3726 proteins were identified between both experimental conditions, with a statistical significance in protein abundance observed between 1260 (q less than 0.20). An examination of these proteins showed marked reduction in pro-proliferative and DNA-replication enzymes in the chemotherapy-treated tumours. Surprisingly, there was a marked absence of apoptotic proteins in both experimental groups. However, there was a significant increase in lysosomal protein abundance following chemotherapy-treatment. In light of this, we believe lysosome-mediated cellular death rather than apoptosis is responsible for the rapid loss of tumour mass typically observed in this model system following treatment. In addition, our findings provide insight into the molecular mechanisms responsible for tumour lysis syndrome.

2. Cancer fatigue progression strongly correlates with cancer cachexia development in advanced non-small cell lung cancer

Seyyed Mohammad Reza Kazemi-Bajestani
Oncology

Introduction: Cancer fatigue is one of the essential components contributing to reduced quality of life. Cancer fatigue is defined as a persistent, subjective sense of tiredness related to cancer and cancer treatment that interferes with usual functioning, and can be recognized as a combination of lack of energy to perform regular activities, social withdrawal, emotional lability and cancer-related physical deconditioning. We proposed that, in addition to other dimensions, cancer fatigue may be related to cancer cachexia, specifically negative energy balance and skeletal muscle atrophy. Methods: Advanced non-small cell lung cancer patients were evaluated twice: a) prior to 1st line palliative carboplatin-based chemotherapy and b) after 4 treatment cycles (about 4 months). At the same time points, cross-sectional computed tomography images were analyzed to quantify skeletal muscle (SM) and total adipose tissue (TAT) loss. Cancer fatigue was measured utilizing The Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) scale. Medical Research Council scale was used to assess dyspnea. Results: Thirty patients [50percentage male; 64.77.6 years] completed the study thus far. Median (interquartile range) of changes (percentage) for fatigue score, SM and TAT were found to be -5.7 (-32.5 +8.6), -4.9 (-12.0 0) and -4.6 (-24.2 +6.9) respectively. Change of FACIT-F-score over time (percentage) was correlated with different features of cancer cachexia [all changes (percentage)]: SM (r=0.49, p=0.007), TAT (r=0.63, p=0.001), total tissue (SM+TAT) (r=0.71, p=0.001). Fatigue worsening was not associated with gender, age or haemoglobin (p larger than 0.05). Change in fatigue score was correlated with increased dyspnea (r=-0.48, p=0.008). Conclusion: Cancer cachexia progression associates with aggravation of cancer fatigue. A loss of muscle would be expected to reduce the capacity for physical functioning. Loss of fat reflects a state of negative energy balance, i.e. defined by energy intake insufficient to cover energy expenditure, which may also be a factor in fatigue.

Oral Presentations

3. Role of Cytomegalovirus Infection on Breast Tumor Growth in Mice

Zelei Yang
Biochemistry

Breast tumors cause local inflammation, which stimulates secretion of autotaxin from tumor-associated adipose tissue. Autotaxin produces lysophosphatidate, which binds to its receptors to promote tumor growth and further inflammatory cytokine production in a vicious inflammatory cycle. Cytomegalovirus (CMV) infects 40-70 percent of cancer-free women, but has been reported in greater than 90 percent of women with breast cancer. CMV infections enter latency but can be reactivated by stress factors. Active CMV infection increases expression of pro-inflammatory cytokines like interleukin 6 (IL-6). CMV in its active and latent states also stimulates the production of anti-inflammatory cytokines like interleukin 10 (IL-10) for self-protection against host immunity. Remodeling of the hosts inflammatory responses by CMV infection could contribute to the vicious inflammatory cycle driven by autotaxin, thus contributing to enhanced tumor progression.We studied how latent CMV infection affects breast tumor progression using female C57BL/6 mice injected with syngeneic E0771 breast cancer cells in the mammary fat pad at 11 weeks after mCMV infection. Tumors and tumor-associated adipose tissues were collected to detect CMV infection, expression of inflammatory mediators including autotaxin, COX-2, and TGFbeta by RT-PCR, and expression of cytokines and chemokines. mCMV infected mice showed suppressed tumor growth for 12 days, but then tumors began to grow rapidly. However, the onset of this growth was unpredictable with delays up to an additional 20 days. No active CMV infection was detected in the samples collected, suggesting that the CMV infection was latent. Autotaxin expression appears to be higher upon CMV infection, suggesting enhanced inflammation. Cytokines including IL-6, IL-9, MIP-1alpha etc. were significantly higher in tumor-associated adipose tissue of infected mice where tumor growth was delayed compared to mice where rapid tumor growth occurred. We are now determining the balance between pro- and anti-inflammatory mediators expressed in infected mice, which might regulate tumor growth.

4. Fusogenic targeted liposomes as next-generation nanomedicine for Prostate Cancer

Jihane Mriouah
Oncology

Chemotherapies for advanced prostate cancer have extended survival, but their efficacy is limited by dose-limiting toxicities due to suboptimal biodistribution. To address the lack of specificity and increase accumulation in Castrate Resistant Prostate Cancer (CRPC), we developed a unique nano-carrier for drug delivery: targeted fusogenic liposomes. We have developed a platform whereby liposomes are formulated with fusion-associated small transmembrane (FAST) protein p14 fused to targeting ligands. The p14 protein induces the fusion of liposomal bilayer with cancer cell membranes to deliver the encapsulated payload directly into the cytoplasm. We have incorporated the ligand bombesin, which allows for targeting to the Gastrin-releasing Peptide (GRPR) that is overexpressed in prostate cancer. We hypothesized that this novel targeted fusogenic liposome formulation would significantly improve the biodistribution and efficacy of chemotherapy. To test this, we modified the clinical liposomal doxorubicin formulation (DOXIL) to incorporate targeted fusogenic p14 protein. We then evaluated the efficacy and biodistribution of these new formulations using in vitro and in vivo models of CRPC. In prostate cancer PC3 cells, p14 or p14-bombesin liposomes deliver doxorubicin inside the cells up to 25 times more efficiently. Additionally, these formulations reduced the IC50 in normally resistant PC3 cells from 21mM to 2mM compared to conventional liposomes. In mice bearing PC3 tumors treated with targeted fusogenic liposomal doxorubicin, we observed tumor growth inhibition of 65% vs non-fusogenic control liposomal doxorubicin. Our results establish a proof of concept for an innovative targeted drug delivery platform that may improve the outcome of patients with CRPC by enhancing the efficacy of approved drugs.

Oral Presentations

5. Mathematical Modelling of Brain Tumour Growth

Amanda Swan
Mathematical and Statistical Sciences

Glioblastomas are a very aggressive and very diffuse type of primary brain tumour. Their diffuse nature, coupled with their proximity to critical brain structures makes effective treatment difficult. Treatment typically involves a combination of surgery and radiation therapy, where the determination of the treatment region boundary is not an easy task. Since it is known that cancer cells infiltrate beyond what can be detected via standard imaging modalities, this boundary must extend beyond the visible tumour mass. In this talk, I will demonstrate how a mathematical model for cancer cell density can assist clinicians in delineating an appropriate boundary for treatment, with the goal of optimizing cancer cell killing, while sparing the most healthy brain tissue.

6. Biomedical Applications of Intense Pulsed Terahertz Radiation

Cameron Hough
Oncology

High-energy ionizing X-rays are commonly used for the diagnosis and treatment of many types of malignant lesions, with more than half of all cancer cases involving radiotherapy. However, ionizing radiation is damaging to normal tissue and is carcinogenic, so exposure potentially leads to toxicity to surrounding healthy tissue and increased risk of developing a secondary malignancy. The terahertz (THz) region of the electromagnetic spectrum occupies a broad band between microwave and infrared radiation, and is non-ionizing. Since THz radiation interacts primarily via vibrational resonances of molecular structures such as proteins and DNA, its mechanism of interaction with biological systems is fundamentally different from that of conventional ionizing therapies. Medical technologies implementing THz radiation for diagnostic spectroscopy and imaging currently exist and have been applied in the early detection of several cancers, as an intra-operative tool during breast cancer surgery, and in other related applications such as pharmaceutical testing and security screening. The effects of THz irradiation on cellular structure and function are beginning to emerge, and recent studies show that exposure of human tissue to intense pulsed THz radiation influences gene expression at both the transcript and protein levels. Furthermore, there is evidence that intense THz pulse exposure might lead to DNA double-strand breaks, as indicated indirectly by assessment of histone H2AX phosphorylation. The onset of this type of damage is thought to be due to a nonlinear resonant response arising from the very high peak electric fields of the THz pulses. Investigation into the threshold at which this cell damage begins to occur will quantify exposure parameters that may provide the same therapeutic potential as ionizing therapies while avoiding many of the deleterious consequences.

Oral Presentations

7. Droplet Digital Polymerase Chain Reaction for Early Detection of Human Papilloma Virus Related Head and Neck Cancer: A Prospective Validation Study

Andre Isaac
Otolaryngology-Head and Neck Surgery

Background: The incidence of oral and oropharyngeal squamous cell carcinoma (OC/OPSCC) caused by oncogenic human papilloma virus (HPV) is rising worldwide. HPV-OC/OPSCC is commonly diagnosed by RT-qPCR of HPV E6 and E7 oncoproteins or by p16 immunohistochemistry (IHC). Droplet digital PCR (ddPCR) has been recently reported as an ultra-sensitive and highly precise method of nucleic acid quantification for biomarker analysis. Objective: To validate the use of a novel, minimally invasive assay for early detection of oncogenic HPV based on oropharyngeal swabs using ddPCR. Methods: This was a prospective cohort validation study of patients with biopsy-confirmed 16 positive OC/OPSCC at our tertiary care Otolaryngology-Head and Neck Surgery referral center. Two groups of controls were used: patients with p16 negaitve OC/OPSCC and patients who underwent tonsillectomy for a benign indication. Each patient underwent an oral/oropharyngeal swab followed by RAN exraction and ddPCR with fluorescent probes to quantitatively detect transcribed oncogenic HPV mRNA for E6 and E7 oncoproteins. Surgical specimens had p16 IHC done according to clinical standards. The sensitivity and specificity of the novel assay was calculated against p16 IHC. Results: Preliminary results are based on 11 recruited patients. Three had p16 positive OC/OPSCC, five had p16 negative OC/OPSCC, while three were healthy controls. All three p16 positive patients tested positive using the ddPCR assay for E6 and E7. None of the 8 p16 negative patients tested positive using the ddPCR assay. The sensitivity and specificity were 100% using 20-50 times less RNA than reported by other studies using RT-qPCR. Conclusion: ddPCR of HPV E6/E7 using oropharyngeal swabs is a rapid and ultra-sensitive method of detecting oncogenic HPV in OC/OPSCC without requiring a tissue biopsy.

8. Autotaxin is an inflammatory mediator and therapeutic target in thyroid cancer

Matthew Benesch
Biochemistry

Introduction - Papillary thyroid cancer incidence has doubled in the past decade, and metastasizes in 40 percent of patients. Of this group, 20 percent are resistant to radioactive iodine treatments. Inflammatory-mediated signaling is correlated to this resistance, but the mechanisms are poorly understood. We provide evidence that lysophosphatidate (LPA) contributes to this problem. Extracellular LPA is produced by the secreted enzyme autotaxin from lysophosphatidylcholine. Autotaxin is important to tissue remodeling, but autotaxin is overproduced in many inflammatory diseases, including cancers. LPA promotes cancer progression, metastasis and therapy resistance. No treatments currently targets LPA signaling. Methods - Autotaxin immunohistochemistry was performed on more than 200 paraffin-embedded patient specimens (primary tumors, benign masses, normal tissue), and LPA/inflammatory mediator concentrations were measured in fresh tissues. The effect of autotaxin inhibition on tumor growth and inflammation in vivo was evaluated in heterotopic xenograft mouse models with 8305C and SW-579 thyroid cancer cells. Results - 1. Autotaxin expression in thyroid cancers was 4-10-fold higher than in benign or normal tissue. 2. Malignant tumors differ from benign tumors by high levels of tumour autotaxin/LPA and inflammatory mediators. 3. LPA increased inflammatory mediator secretion from thyroid cancer cells, which subsequently increased autotaxin secretion. 4. An autotaxin inhibitor in mice with xenograft thyroid tumors decreased tumor volume, angiogenesis and inflammation by 50-60 percent. Conclusions - This study demonstrates that the autotaxin/inflammatory cycle is a focal point for driving malignant thyroid tumour progression and possibly treatment resistance. Inhibiting autotaxin activity provides an effective and novel strategy for decreasing the inflammatory phenotype in thyroid carcinomas, which should complement other treatment modalities.

Oral Presentations

9. EGFL7 inhibits cancer progression by suppressing angiogenesis through its Emilin-like domain

Alisha Kadam
Oncology

EGFL7 is a secreted angiogenic factor that is almost exclusively expressed by endothelial cells. Evidence suggests that EGFL7 expression is elevated in several human tumors. EGFL7 expression is correlated with poor prognosis in malignant glioma, hepatocellular carcinoma, colon cancers, and NSCLC. EGFL7 is comprised of three domains: an Emilin-like (EMI) domain, two Epidermal Growth Factor-like (EGF) repeats and a Matrilin-like (Mat) domain. Recent studies suggest that EGFL7 modulates angiogenesis in the tumor microenvironment through its interaction with Lysyl Oxidase (LOXL2), Integrin Alpha-v Beta-3 and Notch1, but little is known about the mechanistic involvement of its protein domains. Contrary to recent findings, the cancer-specific expression of EGFL7 in two tumor cell lines (HT1080 and MDA-MB-231) led to a significant inhibition of tumor growth in two animal models. The DIVAA assay in mice and the ex ovo angiogenesis assay both showed significant reduction in the blood vessel density for the EGFL7 expressing HT1080 cells (HT0180-EGFL7). Similarly, the in vitro angiogenesis assay showed reduction in the number and length of endothelial cell sprouts (angiogenesis) for the HT1080-EGFL7 cells. Importantly, both the reduction in tumor size and inhibition of angiogenesis was dependent upon the presence of the Emilin-like domain of EGFL7. In summary, we report that the tumor-specific expression of EGFL7 inhibits angiogenesis in in vitro, ex ovo and in vivo models, leading to reduced tumor growth. The Emilin-like domain is essential to the anti-angiogenic activity of EGFL7, suggesting that a potential anti-angiogenic mechanism of EGFL7 lies in this region.

10. Rituximab Monotherapy as Initial Treatment for Post-Transplant Lymphoproliferative Disorder (PTLD)

Hina Vahidy
Medicine

INTRODUCTION PTLDs are a consequence of immune suppression in solid organ transplant (SOT) recipients. Treatment of PTLD is not standardized due to a paucity of high-quality evidence. The role of PET-CT in assessing response to therapy has not been adequately studied. We describe 54 patients treated with reduction of immunosuppression (RIS) and rituximab; chemotherapy was reserved for inadequate rituximab response. The prognostic value of PET scan after initial rituximab in a subset of patients was analyzed. METHODS A database of PTLD cases diagnosed from 1999 to 2013 was queried for those initially treated with RIS and rituximab (n=68). Patients were excluded if they received concurrent rituximab and chemotherapy (n=12) or had primary CNS lymphoma (n=2). Patient and disease characteristics, treatment details and outcome were retrospectively reviewed. Survival was analyzed by Kaplan-Meier and prognostic factors by Cox regression (SPSS). RESULTS 28 (52%) achieved complete remission (CR) or partial remission (PR), and 26 (48%) patients had no response (NR) or progressive disease (PD). 22 patients had PET scan after initial rituximab; 8 (36%) achieved CR, 8 (36%) PR, 1 (5%) NR and 5 (23%) PD. Of the 28 responders, 26 received no further treatment; 7/26 (27%) relapsed and 19/26 (73%) maintained remission. Fifteen (15/54, 28%) received chemotherapy after rituximab; 8 achieved remission, 2 died prior to imaging and 5 progressed. 5-year overall survival (OS) was 52.6%, and 5-year time to progression (TTP) was 52.4%. Several factors were predictive of OS and TTP (Table 1). Poor response to rituximab on PET was predictive of poor OS (HR 6.3, p=0.013) and TTP (HR 8.74, p=0.004). CONCLUSIONS One-third of patients treated for PTLD with RIS and rituximab monotherapy achieved and maintained remission without chemotherapy. PET scan after initial rituximab was predictive of TTP and OS.

Oral Presentations

11. Optimization Models for Practical Implementation in Low-Dose Rate Prostate Brachytherapy

Mustafa Babadagli
Mechanical Engineering

Permanent brachytherapy is a minimally invasive form of radiation therapy used to treat prostate cancer, which involves the permanent implantation of radioactive sources (seeds) inside of the prostate gland. Treatment planning in brachytherapy is based on a decision making process of where to place and how to distribute radioactive sources in order to appropriately target the prostate by delivering an effective dose of radiation to cancerous tissue while sparing the surrounding healthy tissue (especially urethra and rectum). While treatment planning is usually carried out manually by expert planners in the majority of cancer centres worldwide, such a decision making process can also be automated by modeling it as a mixed-integer linear programming (MILP) problem. While there are several research-based and commercial optimization approaches available today for clinical use, many cancer centres find these to be impertinent, too slow, or impractical to integrate into their brachytherapy procedures. In order to fill this existing gap and address the shortcomings of such optimization approaches, we introduce a novel MILP optimization model for interstitial low-dose rate prostate brachytherapy that attempts to mimic the qualities of treatment plans produced manually by expert planners. Our approach involves incorporating a unique set of clinically important constraints, called spatial constraints, into our models. Furthermore, unlike previous optimization studies, we also attempt to capture the essential aspects of the manual-planning method in order to develop an intuitive optimization approach that expert human planners will find seamless to adopt within their daily practice. Preliminary results, obtained from a data set involving ten patients previously treated at the Cross Cancer Institute, show that treatment plans produced through our optimization approach largely capture the qualities and characteristics of manual plans created by expert planners. A highlighting feature of our results is the ability to produce treatment plans in as little as one minute, which is a noteworthy improvement over the currently employed manual methods that usually take about one to four hours by expert planners.

12. Nanomechanical Sensing of Circulating Tumor Cells

Hashem Etayash
Pharmacy, Chemical and Materials Engineering

Detection and analysis of circulating tumor cells (CTCs) at different stages can carry valuable information about cancer. Its a potential biomarker at early stages and a substantial monitor of cancer progression or/ regression at advanced stages. At present, CellSearch system, which is fluorescent and antibody-based, is the only FDA approved and clinically used tool for detection and enumeration of CTCs. With respect to its efficiency, the system still lacks the adequate selectivity and sensitivity as large numbers of metastatic cells do not express the antibody-targeted antigens. The cost associated with application of fluorescent probes, is very expensive and not affordable to some patients. To contribute to the current effort and circumvent these limitations, we developed a label-free, micromechanical array sensor in conjunction with cancer targeting peptides for rapid sensing of circulating tumor cells. To this end, our sensing arrays coated with breast cancer targeting peptide moieties, were able to show statistically significant response to metastatic cancer cells (MCF7 and MDA-MB-231) compared to when exposed to noncancerous cells (MCF10A and HUVEC) in both buffer and blood samples. The arrays have also allowed efficient capture of cancer cells (MCF7) spiked in human blood samples with more than 80% capture yield. Despite that further studies need to be determined, we believe the reported study symbolizes a novel analytical platform that can be a cost-effective and alternative potential for detection and enumeration of circulating tumor cells.

Oral Presentations

13. Accuracy of Resting Energy Expenditure Prediction Equations in Patients with Advanced Lung or Colorectal Cancer

Sarah Purcell
Agricultural, Food and Nutritional Science

Introduction: Determining energy requirements is fundamental for nutritional care. Equations that predict resting energy expenditure (REE, the largest component of total energy expenditure) are often used in clinical settings. The objective of the current study was to assess the agreement between measured and predicted REE (mREE and pREE, respectively) in patients with advanced lung or colorectal cancer. Methods: Patients were recruited from the Cross Cancer Institute. REE was measured by indirect calorimetry and body composition was assessed by dual X-ray absorptiometry. mREE was compared against pREE calculated from 14 commonly-used prediction equations that incorporated anthropometrics and/or body composition (fat-free mass). Agreement between mREE and pREE was evaluated using Bland-Altman analysis, which assesses the bias and limits of agreement between two techniques. Proportional bias was defined as a significant correlation between the bias and the mean difference between mREE and pREE. Results: 67 patients (BMI=26.75.0kg/m2; age 6310 years) had a mean mREE of 1527 290 kcal/day. Compared to equations that used anthropometrics alone, inclusion of fat-free mass in pREE improved prediction of REE as seen in the Wang equation (lowest bias of 3kcal). However, the limits of agreement for this equation ranged from -324 to 328kcal, which translates to -19.1 to 16.9% of the average mREE. All other equations also had wide limits of agreement, ranging from -291 to 258kcal to -764 to 389kcal (a range of 536 to 981kcal of differences). A positive proportional bias was present using the Wang equation (r=0.253, p=0.039). Conclusions: Though prediction capabilities of equations utilising body composition may seem accurate on a group level, there is low agreement between mREE and pREE on an individual level. These findings emphasize the importance of measuring REE when available (especially for those at nutritional risk) to adequately determine energy requirements.

Team Oral Presentations

1. Multicolor Electron Microscopy: Developing the next generation tool-kit for super-resolution microscopy in cell-biology

Christian Foerster and Hilmar Strickfaden
Oncology

Multicolor Electron Microscopy: Developing the next generation tool-kit for super-resolution microscopy in cell-biology. Molecular insights on DNA-repair are crucial for understanding the basic mechanisms of cancer development and cancer therapy. The 2015 Nobel Prize in chemistry recently highlighted the importance of DNA repair. However, many structural aspects of DNA-repair in cancer development and progression (such as DNA-foci formation and changes in chromatin structure) cannot be addressed directly by conventional microscopy and molecular biology due to limitations in light microscopy (resolution) and technical difficulties in mapping distributions of specific biological structures by electron microscopy. To overcome those limitations we propose a unique interdisciplinary approach combining innovative imaging probe design with cutting edge electron microscopy to observe DNA repair mechanisms at the molecular level. Our proposed probe design is based on the synthesis of PAMAM dendrimers decorated with cell-artificial elements like halogens or metal ions as markers for multicolor imaging. The dendritic imaging probes will be used in combination with a transmission electron microscopic (TEM) imaging technique, also referred to as electron spectroscopic imaging (ESI). This technique utilizes the element-specific interactions (inelastic scattering) of highly accelerated electrons emitted from the TEM with the atoms in the biological specimen. We selected fluorine, cerium, and dysprosium as the most promising elements for the development of sensitive imaging probes. Dendrimers of different generations carrying those elements could be identified in the TEM by their size and elemental ESI signature. Dendrimer-based probes will be attached to antibodies targeting DNA repair components, or they will be directly attached to DNA by bioorthogonal click chemistry. We are confident that the novel imaging tool-kit will lead to new groundbreaking discoveries on molecular mechanisms of DNA repair which will ultimately translate into a better understanding of cancer and other diseases.

2. Combining vaccinia virus with guided radiation therapy for the treatment of breast cancer and glioma

Rachel Fung and Brittany Umer
Oncology, Medical Microbiology and Immunology

Vaccinia virus (VACV), the Orthopoxvirus used in the smallpox vaccine, is an ideal oncolytic candidate. We have modified VACV by disrupting components of the virally-encoded ribonucleotide reductase enzyme, rendering the virus dependent on cellular ribonucleotide reductase for dNTP synthesis. These mutations increase the specificity of the virus to replicate in and kill cancer cells, due to elevated levels of these enzymes in tumours compared to normal cells. While promising as a treatment, it is unlikely that oncolytic viruses would be used alone, but rather in combination with existing therapies. In our current study we are investigating whether treatment with radiation therapy (RT) in combination with our modified VACV can provide a superior means of inducing tumour cell death compared to either treatment used alone. Here we show that levels of ribonucleotide reductase are high in irradiated tissue, particularly within the first 48 hours post irradiation. Importantly, oncolytic VACV can replicate and induce cytotoxicity in both breast and glioma cell lines that have been pre-treated with ionizing radiation.. We are now developing targeted radiation treatment protocols with breast and glioma tumor models in vivo using the X-Strahl Small Animal Research Radiation Platform (SARRP). We will investigate whether improved tumour regression can be achieved by combining VACV and RT treatment using the SARRP to deliver image-guided radiation therapy (IGRT) with sub-millimeter precision to tumours, We anticipate that this combinatorial approach of IGRT to de-bulk the tumour, followed by administration of our oncolytic VACV to destroy any residual or infiltrating tumour cells, will result in better clinical outcomes for the patient than using either treatment alone.

Team Oral Presentations

3. Design Concept of Smart Viral Nanoparticles for Targeted Molecular Imaging and Treatment of Prostate Cancer

Anais Martin and Susan Richter

Oncology

Prostate Cancer is the most commonly diagnosed cancer among men in North America. Metastatic prostate cancer, the primary cause of death among prostate cancer patients, may occur early and originate in localized disease that is not histologically detectable. Consequently, there is an urgent need for novel molecular probes to distinguish indolent from aggressive disease and to detect and treat occult metastases. Mammalian virus-based nanoparticles (VNPs) for gene therapy and oncolytic virotherapy are undergoing clinical trials, so the potential of virus-based materials for medical applications has clearly been recognized. Plant viruses can be administered at doses of up to 100 mg per kg body weight without clinical toxicity. Indeed, we have shown previously that Tobacco Mosaic Virus (TMV) can be delivered intravenously, shows good blood compatibility and does not induce hemolysis or coagulation, which make it a promising candidate for molecular imaging and treatment. The goal of the current study was to obtain chemically modified TMV using different synthesis approaches to generate innovative dual TMV-based probes for in vivo optical imaging with fluorescence microscopy and positron-emission-tomography (PET). Tyrosine-based bioconjugation reactions were used to provide exquisite chemoselectivity to the 2130 exposed tyrosine residues per TMV molecule. Coating of the virus particle with polyethylene glycol should lead to enhanced plasma circulation time. In first experiments we attempted to prepare 64Cu-labeled native TMV particles through NOTA complexation using a novel tyrosine-click building block. Other approaches will include (1) a control TMV particle modified with a fluorescent/64Cu dual label and (2) an avb3 integrin-targeting TMV particle decorated with non-RDG peptide H-KTKKVHSQ-NH2, and dually labeled with a fluorescent tag and 64Cu. Here we present our preliminary results on the synthesis and characterization of novel VNPs towards an innovative companion drug nanoplatform for targeted molecular imaging and therapy of prostate cancer.

Poster Abstracts

Pages 12–81

Poster Presentations

1. DLX2 transcription factor expression in the childhood malignant eye tumour retinoblastoma

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Undergraduate Medical Education and Medical Genetics

Introduction: Retinoblastoma (RB) is the most common childhood ocular tumor. In humans, bi-allelic inactivation of the tumor suppressor gene Rb-1 leads to RB, whereas p107, a related tumor suppressor gene, must also be inactivated for the development of RB in mice. DLX2 is a homeobox gene expressed in retinal progenitors as well as retinal ganglion cells (RGC), amacrine and horizontal cells. We are studying the expression of these genes in RB tumours of humans and mice. We expect to gain insight into the retinoblastoma cell-of-origin through analysis of DLX co-expression with retinal cell-specific markers and that DLX2 over-expression will promote differentiation and apoptosis in retinoblastoma cell lines. Methods: Immunohistochemistry was used to study DLX2 expression in formalin-fixed paraffin-embedded sections of mouse and human RB. DLX2 was over-expressed and knocked-down in WERI-1 and Y79 RB cell lines. Real-time quantitative PCR was used to assess GFP-labelled transfected cells for levels of DLX2 co-expression with other markers. Results: DLX2 was expressed in human fetal retina with markers of amacrine and horizontal cells, as well as in childhood and adult retina. Unlike in the mouse, DLX2 was also expressed in the outer nuclear layer of the human retina where the cell bodies of the rods and cones are located. DLX2 was expressed in Chx10:Rb-p107 conditional double-knockout mouse retinoblastoma, in 62/75 (82%) of human RB samples and both RB cell lines examined to date. Dlx2-transfected WERI1 showed increased expression of Brn3b, Brn3a, TrkB and p107 and decreased expression of Olig2, consistent with our findings of these confirmed DLX2 transcriptional targets in our Dlx1/Dlx2 double knockout mouse retina. Conclusions: Co-expression of DLX2 with other progenitor and retinal cell-type specific markers will help provide further insight into the identity of the cell-of-origin of retinoblastoma. Modulating DLX2 expression in human retinoblastoma could promote differentiation and reduce tumorigencity.

2. SHP2 Directly Interact With PD-1 In A Favoured Conformation: A Comprehensive Modelling Study

M. Ahmed, H. Jalily, and K. Barakat
Pharmaceutical sciences

The programmed death 1 (PD-1) pathway is a potent inhibitory mechanism of cytotoxic T cells. Tumors and chronic viral infections can hide from the immune system by overexpressing the PD-1 receptor and its ligands, PD-L1 and PD-L2. Blocking these interactions recently emerged as a game changer approach in cancer and antiviral immunotherapy. Despite the significant therapeutic potential of targeting the PD-1 pathway, very little is known about how these proteins interact and what are the subsequent events that take place following their binding. Recent studies confirmed that following PD-1 binding to either of its ligands, PD-1 recruits the src-homology 2 domain-containing phosphatase 2 (SHP2) to its cytoplasmic ITSM domain. This interaction is responsible for delivering the downstream inhibitory signal resulting from PD1/PD-L1 interaction and ultimately inhibiting T cells activation. Here, we build upon our recent success and continue our efforts toward a complete atomistic model for the full PD-1 pathway. The present study describes, for the first time, a complete homology model for the PD-1 receptor and characterize its interaction with SHP2. Our modeling protocol involved comprehensive protein-protein docking search, exceptionally long molecular dynamics simulations combined with binding energy calculations to explore all potential binding poses between PD1 and SHP2. Our results reveal a favoured conformation for the two proteins to interact and confirm available experimental data for their direct interaction.

Poster Presentations

3. Synergistic Interaction of p53 and Plakoglobin Represses in vitro Growth and Invasiveness

M. Alaee, A. Padda, and M. Pasdar
Oncology

Abstract p53 protein is a master tumor/metastasis suppressor and transcription factor that is either absent or mutated in over 50% of all cancers. p53 regulates various physiological processes from DNA damage response to metabolism. p53 protein structure consists of three domains: a N-terminal domain (NT) with transactivation properties, a core DNA-binding domain (DBD) and a C-terminal (CT) domain with tetramerization and regulatory functions. p53 functions are mediated by posttranslational modifications of these domains and their interactions with different intracellular partners. We have identified plakoglobin (PG) as an interacting partner of p53. PG is a member of the Armadillo family of proteins with dual adhesive and signaling function. Structurally, PG has three domains; a N-terminal a-catenin binding domain, a core of Armadillo (Arm) repeats, which bind various cadherins and signaling partners and a C-terminal transactivation domain. PG is known to act as a tumor/metastasis suppressor and we have shown that it interacts with both wild type (WT) and mutant p53. PGs interaction with mutant p53 restores the WT function in these mutants. These observations suggest that the tumor suppressor activity of PG may be mediated, at least partially, via its interaction with p53. In the current study, PG null and p53 null/mutant carcinoma cell lines were co-transfected with cDNAs encoding various combinations of p53 (WT, NT, DBD, CT) and PG (WT, DN, DArm, DC). Double transfectants were assessed for their tumor/metastasis suppressor activities using in vitro growth, migration and invasion assays. Coimmunoprecipitation studies determined p53-PG interacting domain. Our data revealed that WT-PG and -p53 acted synergistically to significantly reduce growth, migration and invasion rates of transfectants relative to parental cells. The C-terminal domain of PG interacted with the DNA-binding domain of p53 and played a pivotal role in the invasion inhibitory function of PG.

4. Novel role of RYBP in DNA damage repair; implications in cancer therapy

M. Ali, D. McDonald, and M. Hendzel
Oncology

RYBP is a multifunctional protein that plays an important role in various physiological processes including transcriptional regulation, proliferation, differentiation and apoptosis. First discovered as a putative polycomb group (PcG) protein, RYBP was shown to bind to a wide range of proteins including transcription factors, pro-apoptotic proteins, ubiquitin and/or ubiquitinated proteins. Overexpression of RYBP has been observed in Hodgkins lymphoma and glioma. In Hodgkins lymphoma, the expression of RYBP was correlated with therapy-resistance and poor prognosis. On the other hand, loss of RYBP was linked to ERG fusion, a predominant chromosomal aberration in prostate cancer. These findings suggest a possible role of RYBP in genomic stability and/or DNA damage repair. The potential role of RYBP in DNA damage repair was investigated in our study. U2OS cells expressing GFP-tagged RYBP were micro-irradiated and RYBP was found to be rapidly displaced from the DNA damage sites. RYBP displacement is RNF8-depenedent and ubiquitin-dependent. Moreover, overexpression of RYBP interferes with the recruitment of some ubiquitin-dependent repair proteins (e.g. BRCA1 and RAD51) to the irradiation-induced foci around DNA double-strand breaks. Our data indicate that RYBP may unmask binding sites for BRCA1 in DNA damage and its removal from the DNA double-stand break sites might be important for proper DNA damage response.

Poster Presentations

5. A synergistic cytotoxic effect of combination phenylbutazone and hydrogen peroxide on HepG2 cells: Potential involvement of superoxide dismutase

N. Aljuhani and A.G. Siraki
Pharmaceutical Sciences

Introduction: We have previously shown that Cu,Zn-superoxide dismutase (Cu,Zn-SOD) with the presence of hydrogen peroxide and bicarbonate (SOD-peroxidase system) could oxidize the drug, phenylbutazone, culminating in phenylbutazone-carbon centered radicals. The purpose of this study was to investigate if there is a synergistic cytotoxic effect of phenylbutazone and Cu,Zn-SOD-peroxidase system on the hepatocellular carcinoma cell line, HepG2. Methods: To evaluate the cytotoxic effect of combinations phenylbutazone and hydrogen peroxide on HepG2 cells, we measured the metabolic activity as well as the cell viability of HepG2 cells via utilizing both almarblue and trypan blue assays, respectively. Also we used electron paramagnetic resonance (EPR) spectroscopy spin trapping using 5,5-dimethylpyrroline-1-oxide (DMPO) to detect phenylbutazone-carbon centered radical in HepG2 cells. Moreover, we measured SOD activity in the presence and absence of diethyldithiocarbamate (SOD inhibitor). Results: The cytotoxicity of phenylbutazone was synergistically enhanced with the presence of hydrogen peroxide compared with either hydrogen peroxide or phenylbutazone. However, the synergistic cytotoxic effect of combined treatment on HepG2 cells was significantly attenuated by the presence of diethyldithiocarbamate. In addition, the phenylbutazone-carbon centered radicals were detected with the presence of hydrogen peroxide and bicarbonate using intact HepG2 cells; however, the signal of these radicals was significantly decreased by the presence of diethyldithiocarbamate. Also, the activity of SOD in HepG2 cells was significantly decreased by the presence of diethyldithiocarbamate. Conclusion: SOD-peroxidase activity could play a role in phenylbutazone-induced toxicity in HepG2 cancer cells.

6. Regulation of Na+/H+ Exchange in Triple-Negative Breast Cancer Metastasis

S. Amith, J. Wilkinson, and L. Fliegel
Biochemistry

Triple-negative breast cancer (TNBC) is a clinical breast cancer subtype that occurs in 15-20% of patients. It is aggressively metastatic, has high recurrence rates, low responsiveness to chemotherapy, and poor prognoses for patient survival. Currently, no targeted therapies against TNBC exist, so finding novel avenues to fight this disease is imperative. The key pathophysiological role of the Na+/H+ exchanger NHE1 in tumour progression has become clearer in recent years. Hence, the manipulation of pH homeostasis and Na+/H+ exchange in the tumour microenvironment is now being considered a viable anti-cancer therapeutic strategy. We generated key mutations to the NHE1 protein in MDA-MB-231 TNBC cells to determine the role of NHE1 regulation on metastasis. We investigated the effect of changes to NHE1 regulation by: p90RSK/14-3-3 (S703A), ERK1/2 (S766/770/771A or SSSA), and calmodulin (K641R643/645/647E or 1K3R4E). To assess the effect of mutations to NHE1on the metastatic potential of MDA-MB-231 cells, rates of cell migration, invasion, colony and spheroid growth were analysed. S703A cells showed a dramatic difference in morphology, reverting from a mesenchymal to an epithelial-like phenotype with a concomitant loss of expression of mesenchymal marker vimentin. Compared to cells expressing wtNHE1, S703A cells had significantly lower rates of migration, invasion, colony and spheroid growth. Migration and colony formation of SSSA cells were also adversely affected by interfering with the activation of NHE1 by ERK1/2. The 1K3R4E mutant, where NHE1 auto-inhibition and its ability to bind calmodulin were negated, had much higher rates of migration, invasion and spheroid growth. Our data demonstrate a novel link between epithelial-mesenchymal transition and NHE1 regulation that is likely dependent on p90RSK-mediated signalling and binding of 14-3-3. We suggest that Ser703-NHE1 is a critical phosphorylation switch that regulates epithelial-mesenchymal transition in TNBC cells, and is thus a promising target for the development of new chemotherapies.

Poster Presentations

7. Epigenetic Biomarker Expression Analysis in Head and Neck Squamous Cell Carcinoma

C. Andrews, V. Biron, and H. Seikaly
Surgery

Epigenetic modifications are heritable changes in gene expression not encoded in a persons DNA profile. Epigenetic deregulation of cellular processes such as DNA methylation, noncoding RNAs and histone post-translational modifications are included among oncogenic epigenetic modifications. Deregulation of histone methylation, in particular, results in different gene expression profiles within tumours which can then be identified for diagnostic purposes and targeted for reversal with novel therapeutic agents. Among head and neck cancers, oropharyngeal squamous cell carcinoma (OPSCC) incidence has been increasing due to rising rates of human papillomavirus (HPV) related OPSCC. Our aim was to identify the presence of relevant proteins involved in oncogenic epigenetic modifications among OPSCC tumour samples. We focused on enhancer of zeste homolog 2 (EZH2) protein and histone 3 trimethylated lysine 27 (H3K27me3). EZH2s function as a histone 3 lysine 27 methyltransferase plays a role in gene silencing and cell proliferation. In other cancers, overexpression of EZH2 has shown oncogenic actions due to repression of tumor suppresser genes and has been related to malignant potential. Using immunohistochemistry, we confirmed the overexpression of EZH2 leading to increased H3K27me3 presence in the tumour samples compared to the surrounding normal tissue. Future directions include quantifying relative amounts of EZH2, H3K27me3, and cytokeratin present in OPSCC core tumor biopsies of 323 patients using a tissue micro array (TMA) visualized with an Aperio Scanscope system and quantified using HALO image analysis software. Additionally, we will correlate survival outcomes and malignant potential differences based on individual tumor epigenetic profiles and determine differences among these profiles when comparing HPV+ and HPV- patients, as other epigenetic differences between HPV+ and HPV- OPSCC have been established.

8. Characterization of biological features of muscle from cancer patients: preliminary results.

A. Anoveros, A. Bhullar, A. Dunichand-Hoedl, V. Baracos,
A. Rieger, D. Bigam, R. Khadaroo, S. Salim, and V. Mazurak
Agricultural, Food and Nutritional Science

Skeletal muscle wasting has recently been reported to be associated with higher risk of complications during treatment and shorter length of survival in cancer patients. Little is known about the biological characteristics of the muscle during wasting conditions. There are different types of cells residing within the muscle, including myocytes, stem cells of a myogenic lineage, multipotent progenitors, immune cells and adipocytes. Altered proportion and/or interactions between cells within muscle could be potential contributors to skeletal muscle wasting. Computed tomography (CT) images routinely taken during clinical assessment can be used to evaluate body composition (muscle and adipose tissue) in relation to clinical outcomes. The purpose of this study is to characterize biological features of muscle biopsies obtained from cancer patients, where CT derived features of muscle mass are known. We have established methods to characterize stem cell and immune cell populations from muscle samples taken during surgery. Recently established techniques include immunofluorescence microscopy and flow cytometry (FC) have been use to analyze rectus abdominis from patients of same age and sex but who have divergent skeletal muscle areas. Preliminary analysis reveals that patients with the lowest cross sectional area have greater numbers of T cells, neutrophils and macrophages and lower numbers of myogenic progenitors compared with patients with the highest cross sectional area. Ongoing work will identify alterations in other cell populations and will reveal the unique features of muscle wasting.

Poster Presentations

9. Regulation Of NHE1 By BRAF Kinase In Melanoma

A. Augustine and L. Fliegel
Biochemistry

The mammalian Sodium Hydrogen exchanger (NHE) is a ubiquitously expressed membrane protein that extrudes one intracellular hydrogen ion in exchange for one extracellular sodium ion. NHE1 (isoform 1) plays an important role in maintaining intracellular pH (pHi), regulating cell volume and sodium flux in mammalian cells. BRAF kinase is a component of the Mitogen Activated Protein Kinase pathway. It has been implicated in carcinogenesis in melanomas. Our laboratory has previously demonstrated that BRAF (from rat heart extracts) binds to the C-terminal regulatory cytosolic domain of NHE1. Additionally, we have demonstrated that BRAF increases pHi in malignant melanoma cells through the activation of NHE1. In this study, we further characterize the role of BRAF on NHE1 activity. We use inhibitors of NHE1 and BRAF to assess the individual and combined effects on melanoma cell proliferation, migration and invasion in M19 and Mel2A cells. Mel2A cells which endogenously express mutant activated BRAF, proliferate faster than M19 cells which endogenously express wild type BRAF. Using a scratch assay in the presence of the anti-proliferative agent Mitomycin-C, we found that Mel2A cells migrate faster than M19 cells. At 32 hours, there was 100 percent gap closure with Mel2A cells and 40 percent gap closure with M19 cells. In the presence of the NHE1 inhibitor EMD87580, there was a significant reduction in the migration rate of Mel2A cells. At 24 hours, there was 45 percent and 20 percent gap closure with Mel2A and M19 cells respectively. At 32 hours, 60 percent and 25 percent gap closure was observed with Mel2A and M19 cells respectively. Another NHE1 inhibitor, HMA (5-(N,N-Hexamethylene) amiloride), had a similar effect on Mel2A cells. These preliminary results highlight the importance of NHE1 in cell migration and will provide key insights into the molecular mechanisms involving NHE1-BRAF interaction in melanoma cells.

10. An Examination of Radiolabeled Nanoparticles and Their Physiological Effects

S. Berke, T. Purkait, J. Bailey, R. Weberskirch, J. Veinot, and R. Schirrmacher
Oncology

Nanoparticle (NP) technology is emerging as a significant area of interest in various fields of research. Among the numerous uses for NPs are tumor inhibition, cancer imaging, and drug delivery, as well as extensive applications in materials engineering, catalytic use, etc. The physiological effects of NPs are of interest to our group. Currently the effect of NPs on biological functions is relatively unknown and not well investigated. With the recent surge in NP development leading to increasing amounts of NPs in the environment, understanding how NPs interact within living organisms will prove to be invaluable. Labeling NPs with radioactive isotopes, such as fluorine-18, to allow Positron Emission Tomography (PET) imaging in vivo can provide insight into this matter. Our research will investigate the biological distribution and physiological effects of several different NPs: Silicon Nanocrystals (SiNCs) developed by the Veinot Group and self-assembled SiFA-tagged nanoparticles developed by the Weberskirch group (Dortmund, Germany). Techniques to radiolabel and purify NPs with fluorine-18 will be investigated, as well as in vivo PET imaging using radiolabeled nanoparticles (RNPs) in healthy mice. Additionally, injection of drug-derivatized RNPs into tumor bearing mice will be explored to determine tumor affinity and accumulation properties for potential endo-radiotherapy. Barring success of the initial experiments, investigation of RNP serving as drug carriers will be conducted.

Poster Presentations

11. Nuclear localization and possible biological function of matrix metalloproteinase-2 in cardiomyocytes

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Pharmacology and Pediatrics

Matrix metalloproteinases (MMPs) are zinc-dependent proteases known to be involved in extra- and intra-cellular matrix remodeling associated with developmental processes and disease progression. MMP-2 was the first of the 23 human MMPs to be localized to the nucleus. However, the biological functions and substrates of nuclear MMP-2 are mostly unknown. We hypothesized that MMP-2 is present in the nucleus under normal physiological conditions but increases during oxidative stress-induced myocardial ischemia-reperfusion (I/R) injury to proteolyze structural proteins. Lamin A/C, a putative nuclear MMP-2 target, is an intermediate filament protein that provides structural support to the nucleus. We hypothesized that nuclear lamins might be proteolyzed by MMP-2 during I/R injury. Immunofluorescent confocal microscopy and subcellular fractionation showed MMP-2 in both the cytosol and nuclei of neonatal rat ventricular myocytes. Rat hearts were isolated and perfused aerobically by the Langendorff method or subjected to global ischemia followed by aerobic reperfusion in the presence or absence of an MMP inhibitor (100 M o-phenanthroline). Nuclear fractions extracted from rat hearts showed increased MMP-2 activity, but no change in protein level. To identify possible targets, an in vitro proteolysis assay was performed with lamin A or B incubated with MMP-2. Lamin A, but not lamin B, was proteolysed by MMP-2. Troponin I, a known sarcomeric target of MMP-2, was decreased in I/R hearts and this was normalized by o-phenanthroline, demonstrating efficacy of the MMP inhibitor. However, lamin A and lamin C levels remained unchanged in I/R hearts. Nuclear MMP-2 is present in cardiomyocytes under normal physiological conditions, and is increased as a result of I/R injury. This increase of MMP-2 activity in extracts from I/R hearts leads to proteolysis of troponin I, but not lamin A or C. The activation of genes in myocardial I/R injury suggests that other nuclear functions of MMP-2 are likely.

12. Taking advantage of epigenetically induced essentiality at the NMT1 locus to develop a precision medicine approach to treat B cell lymphomas with a N-myristoyltransferase inhibitor

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K. Vincent, R. Lai, W. Dong, J. Arbiser, L. Postovit, M. Lakshmanan, A. Raju,
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Cell Biology

The heterogeneous nature of cancers highlights the need to develop new precision medicines that improve patient selection, therapeutic efficacy, and ultimately survival. This requires drugs for which a robust companion molecular diagnostic identifies those patients with sensitive cancers. Protein N-myristoylation is the covalent attachment of myristic acid (C14:0) to the NH2-terminal glycine residue of numerous eukaryotic and viral proteins, thereafter regulating mainly their membrane targeting. A single myristoyl-CoA: protein N-myristoyltransferase (NMT) isoform catalyses the acylation reaction in yeast, insects, plants and unicellular parasites while there are two isoforms (NMT1 and NMT2) that perform this task in mammals. Using microarray data we identified a drastic reduction of NMT2 expression in a wide variety of cancer cell lines and tumours, this was especially so in hematological cancers. We confirmed this loss at the RNA and protein levels in a selection of B lymphoma cell lines and tumours. We show that NMT2 expression is regulated via epigenetic mechanisms and identified the CpG methylation sites in the NMT2 promoter region. Together, these observations suggested that we could exploit the loss of NMT2 expression in B lymphomas to kill these cells using a synthetically lethal NMT inhibitor (PCLX-001) and leave normal cells, which express both NMTs, unaffected. We show that PCLX-001 selectively kills NMT2 deficient B lymphoma cells in vitro (cell culture) and in vivo (2 cell line derived xenograft models). We next developed a strategy to identify patients with NMT2 deficient DLBCL tumours and establish a patient-derived xenograft (PDX) DLBCL model. Again, treatment with PCLX-001 resulted in complete tumour regression at the higher doses in 7 of 8 mice. Our results provide the first proof-ofconcept for a novel personalized therapeutic approach based on the selective use of a potent NMT inhibitor to treat B cell lymphomas and possibly other cancers.

Poster Presentations

13. Understanding Tamoxifen Resistance In Breast cancer

R. Bekele, G. Venkatraman, R. Liu, X. Tang, S. Mi, M. Benesch,
J. Mackey, R. Godbout, J. Curtis, T. McMullen, and D. Brindley
Biochemistry

Tamoxifen is the accepted therapy for patients with estrogen receptor(ER)-positive breast cancer. However, clinical resistance to tamoxifen, as demonstrated by recurrence or progression on therapy, is frequent and precedes death from metastases. To improve breast cancer treatment it is vital to understand the mechanisms that result in tamoxifen resistance. This study shows that concentrations of tamoxifen and its metabolites, which accumulate in tumors of patients, killed both ER- positive and ER- negative breast cancer cells. This depended on oxidative damage since the breast cancers cells had increased markers for oxidative stress and anti-oxidants rescued the cancer cells from tamoxifen-induced apoptosis. In an adaptive response to the oxidative stress, breast cancer cells induced the expression of Nrf2, which led to the subsequent activation of the anti-oxidant response element (ARE). This increased the transcription of anti-oxidant genes and multidrug resistance transporters. As a result, breast cancer cells are able to destroy or export toxic oxidation products leading to increased survival from tamoxifen-induced oxidative damage. These responses in cancer cells also occur in breast tumors from tamoxifen-treated mice. Additionally, high levels of expression of Nrf2 and its downstream targets ABCC1, ABCC3 plus NAD(P)H dehydrogenase quinone-1 in breast tumors of patients at the time of diagnosis were prognostic of poor survival after tamoxifen therapy. These associations are not predicted from the classical action of tamoxifen through blocking ER? signaling. They thus support our conclusions that tamoxifen-induced killing of cancer cells through oxidative damage is an important component of tamoxifen action. The implication of our finding could have profound outcome in the clinics, as evaluating breast tumors of patients for the expression of Nrf2, ABCC1, ABCC3 and NQO1 could serve as potential predictive markers for tamoxifen response. Moreover, strategies that overcome tamoxifen-induced activation of the ARE could improve the efficacy of tamoxifen in treating breast cancer.

14. Characterization of biological features of muscle from cancer patients: preliminary results.

A. Bhullar, A. Anoveros, A. Rieger, A. Dunichand-Hoedl, S. Salim,
D. Bigam, R. Khadaroo, V. Baracos, and V. Mazurak
Agricultural, Food and Nutritional Science

Skeletal muscle wasting has recently been reported to be associated with higher risk of complications during treatment and shorter length of survival in cancer patients. Little is known about the biological characteristics of the muscle during wasting conditions. There are different types of cells residing within the muscle, including myocytes, stem cells of a myogenic lineage, multipotent progenitors, immune cells and adipocytes. Altered proportion and/or interactions between cells within muscle could be potential contributors to skeletal muscle wasting. Computed tomography (CT) images routinely taken during clinical assessment can be used to evaluate body composition (muscle and adipose tissue) in relation to clinical outcomes. The purpose of this study is to characterize biological features of muscle biopsies obtained from cancer patients, where CT derived features of muscle mass are known. We have established methods to characterize stem cell and immune cell populations from muscle samples taken during surgery. Recently established techniques include immunofluorescence microscopy and flow cytometry (FC) have been use to analyze rectus abdominis from patients of same age and sex but who have divergent skeletal muscle areas. Preliminary analysis reveals that patients with the lowest cross sectional area have greater numbers of T cells, neutrophils and macrophages and lower numbers of myogenic progenitors compared with patients with the highest cross sectional area. Ongoing work will identify alterations in other cell populations and will reveal the unique features of muscle wasting.

Poster Presentations

15. Nodal Contributes To Cisplatin Resistance In Ovarian Cancer Cells

O. Bilyk and L. Postovit
Oncology

The capacity of ovarian tumors to grow and propagate is dependent on a small subset of tumor cells, termed cancer stem-like cells, which contribute to drug resistance, and metastasis. Nodal, an embryonic morphogen has been found to sustain stem cell pluripotency and cellular plasticity, but in cancer cells its expression promotes cancer stem cell renewal, tumor growth, invasion, angiogenesis and metastasis. The role of Nodal in the development of recurrent chemoresistant OC has not been previously investigated. Methods. Cytotoxity of A2780s OC cells to cisplatin was determined by MTT and clonogenic assays. Nodal expression was determined by digital PCR and immunofluorescence staining. To increase Nodal signaling, we used a Nodal expression vector (versus an empty pcDNA3.3 vector; pcDNATM3.3-TOPO cloning kit; Invitrogen). Cell cycle analysis after cisplatin treatment and expression of cancer stem cell marker CD133 were determined using flow cytometry analysis. In vitro sphere limiting diluting assay was applied to measure OC stem cell (OCSC) self-renewal. Results. The expression of Nodal increased significantly in A2780s cells after 24h cisplatin treatment and retained for 96h after cisplatin withdrawal. Overexpression of Nodal rendered A2780s cells more resistant to cisplatin. Exposure of A2780s cells to rhNodal during cisplatin treatment increased the ability of OC cells to form spheres. Cisplatin along with rhNodal treatment increased the population of CD133 positive cancer stem cells. Treatment of A2780s cells with rhNodal prevented cell arrest in S phase after cisplatin treatment (19.4% of cells in S phase after rhNodal+cisplatin versus 63.6% - after cisplatin alone). Conclusion. Our findings demonstrate that Nodal may contribute to cisplatin resistance in OC cells and tumor initiation capacity after drug therapy, and may hold promise as a therapeutic target to prevent chemoresistant recurrence.

16. The Role of Primary Surgery in the Treatment of Advanced Oropharyngeal Cancer

V. Biron, H. Seikaly, H. Zhang, A. Klimowicsz, D. O'Connell,
D. Cote, K. Ansari, D. Williams, L. Puttagunta, and J. Harris
Surgery

Background: The treatment paradigms for advanced oropharyngeal squamous cell carcinoma (OPSCC) are controversial and continuously evolving. Treatment guidelines suggest that primary surgery with adjunctive therapy or chemoradiation are acceptable treatment regiments, but there are no definitive literature comparing the survival outcomes of these widely varied approaches. The purpose of this study was to compare differences in survival of patients with advanced stage OPSCC according to surgical and non-surgical treatments, when stratified by smoking and P16 status. Study design: This is a prospective longitudinal population-based study Methods: All patients diagnosed with advanced OPSCC were included. Patients were classified as smokers if they had a tobacco use history 10 pack years and all patients with advanced stage OPSCC had their p16 status determined through analysis of their preserved tissue. Results: There was no significant DSS difference between combined modality treatment groups in non-smokers that had p16 positive cancers but in smokers with p16 positive cancers the DSS for S+CRT was significantly higher then CRT. Patients who had p16 negative cancers that had highest DSS when treated with S+RT/CRT. Multivariate Cox regression analysis showed that increasing ECOG score, smoking, p16 status, higher stage, and treatment with surgery protocols were significant determinants of survival. Conclusions: S+CRT offers the highest survival advantage in smokers with p16 positive cancers while all combined treatment modalities offer equivalent survival outcome for patients who are non-smokers with p16 positive cancers. S+RT/CRT offers a significant survival advantage over CRT in patients with p16 negative cancers.

Poster Presentations

17. Molecular Imaging of Cancer

B. Blasiak, F. Veggel, A. Abuldrob, and B. Tomanek
Oncology

Within the program, a multi-disciplinary group develops multi-modal contrast agents for MRI, PET and optical imaging of the brain, breast, prostate and lung cancers. We are using nanoparticles, radionuclei as well as infrared probe conjugated with the tumor specific antibodies and use them for in vivo MR, PET and optical imaging of tumors in animal models with increased specificity and sensitivity when compared to such imaging techniques using non-targeted contrast. Because of the interdisciplinary nature of the challenges, a team of experts has come together with complementary range of expertise. We brought together top specialists from different fields: molecular biology, cell biology, physics, nanotechnology, breast oncology, animal and clinical imaging. The studies are performed by the specialists from across Canada: Ottawa (contrast agents synthesis), Montreal (IR and toxicology), Calgary and Edmonton (MRI), Victoria (nanoparticles production), Vancouver (PET). The successful development of the contrast agents for breast cancer patients will 1) ensure earlier and more accurate diagnosis, 2) provide a guide for individualized therapies to improve outcome, and 3) allow treatment monitoring. The developed technology could be extended to other cancers in future research. The results of our studies and future program directions will be presented.

18. Clinical Set-Up for PSMA imaging in prostate cancer and evaluation of novel imaging probe

V. Bouvet, M. Wuest, J. Bailey, R. Schirrmacher, and F. Wuest
Oncology

Prostate Cancer (PCa) is the second most lethal cancer in men and prostate-specific membrane antigen (PSMA) is one of the most attractive targets for molecular imaging of PCa. To date, the lysine-urea-glutamates (LuG)-containing compound [18F]DCFPyl, a compound developed by Pomper et al. at John Hopkins Medical School, has entered into the clinic as an 18F-labeled radiotracer for PSMA imaging. However, despite its promising in-vivo biodistribution and uptake, the synthesis of [18F]DCFPyl suffers from extremely low production yields. The purpose of our present collaborative project is two-fold. On one hand, improve the synthetic scheme and the production yields for the automated synthesis of [18F]DCFPyl and on the other hand, evaluate alternative low molecular weight (LuG)-containing compounds. In order to obtain an easy, high yielding and reproducible synthesis for clinical use, we developed a novel methodology to produce [18F]DCFPyL. We optimized our synthesis using the widely available single reactor GE TRACERlab FXFN, with a 2 step, 1 precursor process which affords [18F]DCFPyL with 20-30% radio decay-corrected yields compared to the 2% presently described in literature. Concurrently, a series of 6 fluorinated LuG small molecule PSMA inhibitors with promising pharmacokinetic properties were synthesized. Their IC50, lipophilicity, pharmacokinetic characteristic and in-vivo stability were evaluated using LNCaP cell based assay, [18F] Dynamic Positron Emission Tomography and organ biodistribution. Synthesis of the radiotracer [18F]DCFPyL was accomplished in good radiochemical yield and purity using an automated synthesis unit. The favorable radiopharmacological profile and automated synthesis are compatible with future clinical applications of this 18F-labeled PSMA imaging agent. Furthermore, one novel [18F]LuG analogs exhibits superior uptake than the previously described [18F]DCFPyL and full pharmacological evaluation of this analog is under way.

Poster Presentations

19. The Global Landscape of Cellular Immunotherapy Clinical Trials

Z. Breckenridge, K. Bonter, and T. Bubela
Health Policy and Management

Cancer remains one of the leading causes of morbidity and mortality worldwide, with the number of new cases expected to rise by 70% over the next two decades. Cellular immunotherapy (CI) shows curative potential for patients with both solid tumors and hematoma. We describe the global landscape of CI trials derived from clinical trial registrations (CTR) and publications. We analyze trends in CI to predict future development patterns, expected efficacy, business models, manufacturing requirements, clinical trial designs, and clinical applications. We sampled trials registered between 1992 and September 2014 from the four largest clinical trial registries worldwide. In consultation with CI experts, we developed a search algorithm which yielded 16, 593 unique CTRs. Inclusion/exclusion criteria were applied, resulting in a dataset of 1363 CTRs. We then manually reviewed each registration recording information including: cell type, ex vivo manipulations, manufacturing processes, dose, co-interventions, and industry affiliation. Of the 1363 trials in our dataset, 556 trials are expected to finish between 2014 and 2017. The number of trials employing active immunization approaches was superseded in 2012 by trials employing passive approaches. Trends indicate that CI interventions are increasing in specificity in terms of antigenic target, and complexity in ex vivo manipulation. Clinical CI research is progressing rapidly. The next steps will be to employ these data in cost effectiveness models to ensure CI is developed in a manner that health systems can afford.

20. Effects of human platelets on lung cancer stem cell invasion

M.J. Yan, A. Radziwon-Balicka, and P. Jurasz
Pharmaceutical Sciences

Purpose. The cancer stem cell theory of cancer origin suggest a small population of cancer cells has stem cell-like characteristics (CSCs) and is responsible for initiating new tumors following metastasis. Studies have shown platelets contribute to metastasis, in part by stimulating cancer cell migration via release of chemokines from platelet granules, such as stromal derived factor-1a (SDF-1a). SDF-1a is known to mobilize both bone marrow and cancer stem cells via increased matrix metalloproteinase (MMPs) expression. Hence, we hypothesize that activated platelets preferentially induce CSC migration by releasing SDF-1a which binds to its receptor CXCR4 on CSCs leading to increased MMP production and invasion. Methods. The invasion of Hoechst 33342-negative side population (SP) identified CSCs MMP-dependent invasion as compared to total A549 population were measured via a modified Boyden Chamber assay in response to collagen activated human platelet relesates. Invasion of CD133 identified CSCs was analyzed by comparing the amount of CD133 positive CSCs vs. CD133 negative cells that invaded the Boyden chamber under fluorescence microscopy. Results. Platelet releasates preferentially promoted SP identified A549 CSCs invasion. AMD3100 (10uM) inhibited total but not A549 SP cell invasion. CD133 identified CSCs did not show difference between the number of invasions of either population with resting or activated platelet relesates. Conclusions. Activated human platelets preferentially stimulate the invasion of SP-identified, but not CD133 identified, CSCs within the A549 cell line. Further experiments are required to delineate the role of SDF-1a-CXCR4-MMP signalling in platelet-stimulated cancer stem cell invasion.

Poster Presentations

21. Nuclear Factor I Regulates Expression of HEY1 in Malignant Glioma

M. Brun and R. Godbout
Oncology

Grade III and IV astrocytomas, commonly referred to as malignant glioma (MG), are the most common adult human brain tumour. Despite aggressive treatment including surgery, radiation, and chemotherapy, median survival remains less than two years. The nuclear factor I (NFI) transcription factor family (NFIA, B, C, and X) is normally expressed in the developing brain and promotes glial cell differentiation. NFI is also expressed in MG, where it regulates expression of glial genes, and genes involved in proliferation and migration. We used chromatin immunoprecipitation (ChIP)-on-chip with a promoter microarray to identify additional NFI target genes in MG cells. We identified 403 putative NFI target genes, including HEY1, a Notch effector gene that promotes maintenance of undifferentiated cells in the developing brain. Using electrophoretic mobility shift assays, we show that NFI binds to NFI consensus binding sites in the HEY1 promoter. Knockdown of NFIs in in conjunction with reporter gene assays and quantitative PCR (qPCR) reveal that NFI represses expression of HEY1 in MG cells. We also examined expression of glial genes, including NFIs following HEY1 knockdown, as HEY1 promotes maintenance of undifferentiated cells. Knockdown of HEY1 in MG cells resulted in increased expression of GFAP, a marker of glial cell differentiation. When we examined expression of HEY1 MG biopsy cells cultured under neurosphere conditions, we observed a decrease in NFI, and concomitantly, an increase in HEY1 expression. Taken together, our results demonstrate that NFI represses expression of HEY1 in MG, and that expression of HEY1 and NFI in MG tumours may regulate glial cell differentiation within these cells.

22. Self-renewal capacity of sensory organ precursor cells

J. Ayeni, A. Audibert, P. Fichelson, M. Srayko, M. Gho, and S. Campbell
Biological Sciences

Developmentally regulated cell cycle arrest is a fundamental feature of neurogenesis that is thought to be important for coordinating cell division with correct cell fate determination. The precise role that quiescence plays during neural development remains poorly understood, however. Moreover, growing evidence links defects in neural quiescence to neurodegenerative disease and tumour formation in humans. To study this phenomenon we examined thoracic sensory organ development, seeking to understand how the regulation of G2/M timing is coordinated with neuronal cell fate specification. Phenotypic analysis and time-lapse imaging of the thoracic microchaetae lineage showed that forcing sensory organ precursor (pI) cells to divide prematurely resulted in production of supernumerary external sensory organ cells. These supernumerary cells did not arise from defects in segregation of cell fate determinants that were previously shown to cause similar phenotypes. Instead, regulation of G2 phase quiescence ensured that neural progenitor cells did not undergo self-renewal before reception of a developmental signal that promotes neuronal differentiation. The cell cycle arrest mechanism that regulates the timing of mitosis is therefore important for synchronizing pI progenitor cell division with signals that potentiate neuronal cell fate, during development of a sensory organ lineage.

Poster Presentations

23. Seeking intracellular targets of myocardial matrix metalloproteinase-2 activation in doxorubicin cardiotoxicity

B. Chan, A. Roczkowsky, B. Hughes, and R. Schulz
Pediatrics and Pharmacology

Doxorubicin (DXR) is a commonly prescribed antineoplastic agent in many cancer chemotherapeutic regimens. However, its side effects cause heart failure in some patients. Studies in rats have shown that DXR treatment increases matrix metalloproteinase-2 (MMP-2) levels in the heart. MMP-2 is an extra- and intra-cellular protease that plays an important role in heart diseases associated with increased oxidative stress. Intracellular MMP-2 may play a causative role in DXR cardiotoxicity by proteolysing sarcomeric proteins in cardiomyocytes. Human fibrosarcoma (HT1080) cells and neonatal rat ventricular myocytes (NRVM) were treated with DXR (0.5 ?M) MMP inhibitors ARP-100 or ONO-4817 (1 ?M each) for 2-48 hr at 37C. 24 hr DXR caused 15% cell death in HT1080 cells but none in NRVM as assayed by lactate dehydrogenase release (n=8). 36-plus hr DXR was toxic to NRVM. In NRVM, 12 hr DXR increased oxidative stress as evidenced by a 20% reduction in aconitase activity (n=8) and activated intracellular MMP-2 by 3x relative to vehicle (n=5). ARP-100 or ONO-4817 attenuated 24 hr DXR-induced MMP-2 activation by 60% (n=7). Intracellular MMP-2 activation was partly accounted for by a two-fold increase in MMP-2 protein levels (n=4). 24 hr DXR reduced troponin I levels by 40%, which was partially prevented with ARP-100 (n=5). DXR, at a clinically relevant concentration, acutely activates myocardial MMP-2 via, in part, increased MMP-2 protein level in association with increased oxidative stress. DXR-induced MMP-2 activation resulted in troponin I proteolysis. Future experiments in mice will study the effect of DXR on cardiac function, MMP-2 activity, and identify further MMP-2 substrates. Understanding the mechanism of DXR-triggered myocardial injury via MMP-2 will determine whether blocking intracellular MMP-2 activity is a possible adjuvant therapy in patients receiving DXR chemotherapy.

24. The A1298C polymorphism in the methylenetetrahydrofolate reductase gene is a risk factor for deep vein thrombosis in pediatric cancer patients.

W. Choi, K. Dietrich, M. Spavor, S. Israels, J. Halton, E. Shereck, and L. Mitchell
Pediatrics

Introduction: One third of pediatric cancer patients will develop a deep vein thrombosis (DVT) during their cancer treatment. The occurrence of DVT is associated with significant morbidity and mortality. As not all children will develop DVT, determining biomarkers that indicate which children are at risk is important in order to optimize clinical care. Elevated plasma homocysteine levels are a risk factor for DVT in adults. Two single nucleotide polymorphisms (SNPs) in the MTHFR gene have been demonstrated to cause an elevation in plasma levels of homocysteine. The objective of the study was to determine the association of DVT with genetic polymorphisms in the MTHFR gene in pediatric oncology patients. Methods: We performed a multicenter cross-Canada case control study. Survivors of childhood cancer who experienced DVT while undergoing cancer treatment (cases) were matched with survivors of childhood cancer who did not experience DVT (controls). We recruited 369 patients (109 patients with DVT and 260 controls without DVT) and 104 normal controls. An r2 of 0.8 for linkage disequilibrium and a MAF greater than 5% were used as threshold values for SNP selection. We identified 8 tagging SNPs including rs1801131: A1298C and rs1801133: C677T. Analysis of genetic polymorphisms was done by allele specific primer extension. Results: Frequencies of the 1298CC genotype were 13.7% in patients with DVT as compared to 7.1% in patients without DVT (OR=2.09; 95% CI:0.99-4.4, P=0.052). The frequency of the 1298CC genotype between the normal controls and all cancer patients was similar (OR=1.07; 95% CI:0.49-2.34, P=0.853). Conclusions: We have identified an association between DVT and A1298C polymorphism in childhood cancer patients. Acknowledgments: WSC is supported by AIHS summer student award and the study was funded by the Hair Massacure Foundation and CIHR.

Poster Presentations

25. The Alberta Prostate Cancer Research Initiative (APCaRI) Prostate Registry and Biorepository

C. Vasquez, A. Fairey, B. Donnelly, and J. Lewis

Oncology

Background: The APCaRI Registry and Biorepository is an Alberta-wide prospective study to collect comprehensive demographic, clinical and outcomes information from a large cohort of patients who are at risk or have a diagnosis of prostate cancer, and pair this with a state-of-the-art biorepository. Our mission is to enable team members and the broader research community to apply next generation genomic, proteomic, metabolomic and transcriptomic analyses to the development of new and better tests for prostate cancer (PCa). During the next 5 years we will recruit more than 5,000 participants in Alberta. Aims: To collect, process, store and share biospecimens and demographic, patient reported outcomes and clinical information from participants with pre-clinical and clinical prostate pathology. Methods: 1. Patients with pre-clinical and clinical prostate pathology are identified by clinician at treatment centres across Alberta. 2. 68 aliquots of serum, plasma, buffy coat, red blood cells, urine and semen are obtained at intake and each year for 5 years. Processing time from body to freezer is 2 hours or less. 3. Comprehensive demographic, co-morbidity, family history and quality of life information are stored in our REDCap database and updated every six months. Cohorts Available: 1. Pre-diagnosis: Biospecimens from 586 participants are available for research. 290 have confirmed diagnosis of PCa, 187 have negative biopsies, 27 pending. Mean age at enrollment 62 (SD=8.23, range 35-87). 2. Post-diagnosis: Biospecimens from 426 participants are available for research. Mean age at enrollment 67.4 (SD=8.37, range 41-89). By 2019, the APCaRI Registry and Biorepository will represent the largest, most comprehensive prostate cancer registry and biorepository in the world. This will enable the Albertan research community and investigators worldwide to conduct a wide array of high impact clinical, epidemiological and biological studies on large, well-validated cohorts supporting a transformative impact on those living with prostate cancer.

26. Vaccinia Virus Lacking F4L Gene Shows Excellent Safety and Potent Oncolytic Activities in Treating Breast Cancer

S. Chaurasiya, N. Favis, C. Irwin, K. Potts, R. Noyce, D. Evans, and M. Hitt

Oncology

We have generated mutant vaccinia virus (VV) deleted in F4L or J2R or both genes which are involved in dNTP synsthesis. VV, a DNA virus with large genome, needs large pool of dNTPs for efficient replication. dNTPs concentrations are much higher in rapidly dividing cancer cells compared to normal cells. Therefore, we hypothesised that these F4L-mutant VVs will show cancer selective replication.We tested the specificity of these viruses in vitro in monolayer and spheroid cultures of breast cancer and number cells. As expected, the F4L-mutated VVs were found to replicate to high levels, much like the wild type virus, in cancer cells but in the slow dividing normal cells mutated VVs were highly attenuated. We also tested the safety and anti-tumor efficacy of our mutant viruses in xenograft and syngeneic mouse models of breast cancer. The F4L-mutated VVs show excellent safety (almost no replication in normal tissue) and significantly delay tumor growth ultimately prolonging the survival of treated mice compared to the control group. Overall, our finding suggest that VVs deleted in F4L gene holds promise as oncolytic therapy for braest cancer.

Poster Presentations

27. Docosahexaenoic acid-related inhibitory mechanisms in malignant glioma cell migration

X. Xu and R. Godbout
Experimental Oncology

Malignant glioma (MG) is the most common primary malignancy in the central nervous system. Brain fatty acid binding protein 7 (FABP7) has been reported to be associated with increased MG cell migration and a poor clinical prognosis. Long chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and arachidonic acid (AA) are abundant in brain cell membranes. DHA is a natural ligand of FABP7 and certain nuclear receptors including peroxisome proliferator-activated receptor gamma (PPARγ), a tumour suppressor gene involved in regulation of lipid metabolism and cancer cell migration. DHA supplementation has been shown to inhibit tumour cell growth in vitro and tumour formation in vivo. This study was designed to investigate whether DHA plays an inhibitory role in MG cell migration and infiltration through alteration of MG related lipid metabolism and through FABP7-mediated nuclear transportation with subsequent PPARγ activation. We used stable U87 control and U87 FABP7-transfected MG cell lines as models to test the effect of FABP7 and DHA on the expression of genes involved in lipid metabolism in MG cells. Cells were treated with bovine serum albumin (BSA) vehicle, 60 μM AA or 60 μM DHA for 24 hours To date, we have shown that DHA treatment significantly decreases the levels of cytochrome P450 family member CYP2J2, a key enzyme in AA cytochrome P450 metabolism, but only in U87 FABP7 transfected cells. Our combined immunofluorescence data and and nuclear/cytosol fractionation results indicate that both AA and DHA treatment can induce PPARγ nuclear expression. Our results to date suggest a dynamic FABP7-mediated shuttling of PPARγ from the cytoplasm into the nucleus as a function of AA or DHA treatment. We will pursue our analyses by investigating the effect of FABP7 and fatty acids on other nuclear receptors and lipid metabolism proteins.

28. Linking Mutations to BRG1 to Aggressive Dedifferentiated Endometrial Carcinoma

M. Coatham, X. Li, C. Lee, and L. Postovit
Obstetrics and Gynecology

Biallelic inactivation of BRG1, one of the core catalytic subunits in the eukaryotic SWI/SNF chromatin remodeling complex, as result of nonsense, frameshift and splice site mutations has been reported in non-small cell lung cancer (NSCLC) and small cell carcinoma of the ovary, hypercalcemic type (SCCOHT). Both NSCLC and SCCOHT share a common rhabdoid phenotype. Recently, endometriod adenocarcinomas displaying an undifferentiated, large cell, rhabdoid phenotype have been discovered and reported to be BRG1-deficient. This dedifferentiated endometrial carcinoma (DDEC) is a highly aggressive type of endometrial cancer. Understanding the contribution of BRG1 inactivation to the DDEC phenotype and biology, will allow for targeted therapy to be developed against the unique molecular features of this cancer. This study could also provide insight into how BRG1 inactivation maintains undifferentiated gene expression programs in other cancers such as NSCLC and SCCOHT. Targeted sequencing together with immunohistochemistry was utilized to detect mutations and loss of BRG1 in clinical cases of DDEC. Established human endometrial cancer cell lines were characterized for their level of BRG1 expression by pairing immunofluorescence studies with Western blot analysis. The expression of EMT and stem cell-related genes in these cells lines was determined using qRT-PCR. Close to 40% of clinical cases of DDEC show a loss of BRG1 with the majority having developed in a mismatch repair protein-deficient molecular context. Most of the highly microsatellite unstable, hypermutated (MSI-high) endometrial cancer cell lines expressed BRG1 at low levels and retained an epithelial-like phenotype. These results lay the foundation for developing an in vitro model of DDEC using current CRISPR technology to knockout BRG1 in MSI-high endometrial cancer cell lines. Direct comparison between the in vitro derived human DDEC model and patient derived xenograft (PDX) models from clinical DDEC cases will be critical to our understanding of the role of BRG1 in cellular dedifferentiation.

Poster Presentations

29. Oligomerization of Equilibrative Nucleoside Transporter 1

X. Cravetchi, G. Vilas, and J. Hammond
Pharmacology

Equilibrative nucleoside transporter subtype 1 (ENT1) is a ubiquitously expressed membrane transporter in mammalian cells. It is critical in regulating biological activities of endogenous nucleosides such as adenosine, as well as chemotherapeutic nucleoside analogues such as gemcitabine, a pancreatic cancer drug, and cytarabine, a leukemia pro-drug. The expression level of hENT1 has also been identified as a biomarker to assess an individuals potential response to chemotherapy. Despite the importance of this system to chemotherapy, little is known about how its expression and function is regulated. It is becoming increasingly apparent that many membrane proteins function as oligomers, and the oligomeric state can influence protein function. There have been no reports to date on whether ENT1 can form dimers. The current study was conducted to determine whether ENT1 can self-associate, and potentially function as higher oligomeric structures. Using biochemical and immunofluorescence techniques, our lab has shown that hENT1 can form dimers in intact cells. Co-immunoprecipitation in HEK293 cells of MYC- and HA-tagged hENT1 showed the possibility of association as the pulldown of protein with one epitope successfully brought down the alternatively tagged protein. PK15-hENT1 cells were subsequently transfected with MYC-hENT1 and the interaction between the proteins was quantified using a proximity ligation assay. Anion exchanger 1 (AE1) and carbonic anhydrase II (CAII) were used as a positive control, ENT1 and glucose transporter 1 (GLUT1) were the negative control pair. Interaction between FLAG- and MYC-ENT1 occurred approximately 505 +/- 101 times per cell, statistically similar to AE1/CAII with 540 +/- 157 signals per cell. In contrast, hENT1 and GLUT1 interactions occurred only 39 +/- 9 times per cell. Following these studies, we conclude that hENT1 can exist in a dimeric state, opening a new avenue of research relating to regulation of transporter activity in response to therapeutic application.

30. Potentially effective treatment for granulosa cell tumour through combination of two apoptosis inducing agents, PAC-1 and TRAIL

P. Crosley, K. Agopsowicz, M. Weinfeld, and M. Hitt
Oncology

Granulosa cell tumour (GCT) is an uncommon form of ovarian cancer, constituting 5 percent of ovarian neoplasms. While 5-year survival of early stage GCT is over 90 percent, 30 percent of women will experience relapsed disease and 80 percent of them will die of disease. Evasion of apoptosis is one hallmark of cancer. Caspase-3 (CASP3) is at the hub of multiple apoptotic pathways and, when active, contributes to an irreversible cascade of protease activity leading to programmed cell death. Small-molecule drug procaspase activating compound-1 (PAC1) induces activation of CASP3 by sequestering an inhibitory zinc ion. It has been shown, in vivo, to be well-tolerated and effective in the treatment of various cancers. This study reports on preliminary experiments testing our hypothesis that combining small-molecule PAC1 with TNF-related apoptosis-inducing ligand (TRAIL), which can induce the extrinsic apoptotic pathway, will result in increased killing of GCT cells, versus either drug alone, and may represent a novel therapeutic approach. GCT cell line, KGN, was independently treated in vitro with a 6-log range of PAC1 and TRAIL concentrations to establish dose-response curves. Calculated EC50 values guided selection of concentrations for PAC1 and TRAIL which were applied in various combinations to look for improved efficacy. Preliminary in vitro tests show 20 uM PAC1 reduces viability in KGN cells, compared to untreated control. Likewise, 10 ng/mL TRAIL reduces KGN viability compared to control, although PAC1 alone was significantly more effective than TRAIL alone. Combination of 20 uM PAC1 with 10 ng/mL TRAIL produced significant decrease in cell viability compared to control, PAC1 alone, and TRAIL alone. These in vitro results support the hypothesis that combining small-molecule drugs targeting CASP3 in combination with drugs affecting the apoptotic pathway is potentially an effective, novel treatment for GCT that warrants further study.

Poster Presentations

31. Central neurons that regulate appetite are changed in tumor-bearing animals possible link to cancer-associated wasting (cachexia)

B. Doslikova, A. Dunichand-Hoedl, D. Marks, V. Baracos, and W. Colmers

Pharmacology

Cachexia is a syndrome characterized by the loss of appetite, fat and muscle seen in chronic illnesses, including heart failure, obstructive pulmonary disease and cancer, and affects over 9,000,000 patients globally. Cachexia reduces quality of life, the ability to respond to and tolerate therapies, and survival. In cancer, cachexia afflicts 50-80% and kills 30% of all patients. The mechanisms underlying cachexia are poorly understood and good treatments are lacking. Pathophysiology of fat and muscle is generally studied as a cause of cachexia, while the brain which regulates all biological processes has been largely ignored. We address this crucial gap by investigating if and how properties of brain neurons, specifically those that regulate appetite and affect fat and muscle, might be altered in cachexia caused by experimental tumours implanted into otherwise healthy rats. In animals with a tumour-associated loss of appetite, neurons from a brain region known to regulate appetite behave differently than in controls. While some such neurons are more active and less sensitive to inputs from other neurons, the exact opposite is seen in different types of neurons in the same region. Thus, the presence of tumour-mediated cachexia coincides with dramatic changes in the basic properties of brain cells which govern appetite and influence muscle and fat. Conceivably, tumour-derived signals may cause the behavioural changes of these cells, in turn inducing cachexia; a hypothesis we are currently pursuing. Collectively, our findings increase our understanding of long-neglected central pathophysiology of cancer cachexia and could lead to novel treatments.

32. Imaging glucose and fructose metabolism in breast cancer: A mouse study

I. Hamann, M. Wuest, V. Bouvet, A. Marshall, O. Soueidan,
F. West, C. Cheeseman, and F. Wuest

Oncology

The role of [18F]FDG-PET in clinical breast cancer (BC) imaging is still limited since up to 50% of BCs do not express high levels of hexose transporter GLUT1. An interesting alternative target may represent fructose transporter GLUT5. It is elevated in several types of cancer, including BC. The goal of the present study was to analyze uptake characteristics of selected fructose and glucose-based PET radiotracers in a murine BC model in vitro and in vivo. Dynamic PET imaging studies confirmed radioactivity uptake but no trapping of fructose-based radiotracers. While 1-[18F]FDF revealed low uptake levels, uptake of 6-[18F]FDF was significantly higher but was followed by slight a washout over 2 h. 6-[18F]FDG reached maximum uptake at 20 min with no further accumulation over time. Clinical glucose radiotracer 2-[18F]FDG showed continuous increase of uptake. In EMT-6 tumors, GLUT5 mRNA expression was around 20,000-fold lower compared to GLUT1. Comparison of GLUT mRNA levels in EMT-6 tumors with mouse muscle revealed, that mouse muscle also shows higher GLUT5, similar GLUT2 and lower GLUT1 mRNA levels (GLUT1 50:1; GLUT2 1:1.6; GLUT5 1:6). Determination of GLUT5 protein levels, however, exhibited higher expression levels in tumor versus muscle tissue. Uptake of fructose-based PET radiotracers cannot simply be correlated with GLUT5 mRNA levels but nicely correspond to GLUT5 protein levels. Taken together, our results point out the importance of detailed biochemical assessment in combination with functional studies to characterize molecular signatures of breast cancer in preclinical studies.

Poster Presentations

33. Can Gamma Delta T Cells Target Breast Cancer Stem Cells?

I. Dutta, A. Medina, L. Postovit, and G. Siegers
Experimental Oncology

Gamma Delta T cells (GDTc) are immunosurveillance cells comprising 2-5% of circulating lymphocytes. GDTc target recognition and lysis are mediated by the T cell antigen receptor (TCR) and/or the natural killer receptor NKG2D. Among others, GDTc respond to self-molecules signalling cellular stress such as UL16-binding proteins (ULBP) 14 and MHC-like proteins MICA and MICB, which are often upregulated on transformed cells. Phase I clinical trials have confirmed the safety of GDTc cancer immunotherapy. However, little has been done to determine whether GDTc can target cancer stem cells (CSC), the small population of cells responsible for tumor maintenance, therapy resistance and recurrence. Thus far, only two studies have reported GDTc targeting CSC in colon and ovarian cancers. We are investigating whether GDTc target breast cancer CSC. Our panel of human breast cancer cells shows a range of CSC percentages, defined by the cell surface signature CD44+CD24-. Cytotoxicity experiments have suggested an inverse correlation between GDTc cytotoxicity and prevalence of CSCs in breast cancer targets. Using the SUM 149 cell line as our model, we are sorting CSCs via flow cytometry and monitoring the kinetics of reversion back to the original heterogeneous population. Cytotoxicity experiments at defined time points will reveal the ability of GDTc to target CSCs. We are examining ULBP1-4 and MICA/B expression levels in CSCs vs non-CSC SUM149 cells. We hypothesize that lower tumour antigen expression on CSCs, potentially due to epigenetic regulation, enables their escape from GDTc killing. Our ultimate aim is to reverse this process.

34. Adipose tissue alterations in an animal model of colorectal cancer: effects of tumour, chemotherapy and fish oil intervention

M. Ebadi, K. Giles, and V. Mazurak
Agricultural, Food and Nutritional Science

Dietary fish oil (FO) has potential to prevent weight and muscle loss and improve response to chemotherapy in a neoplastic state. In this study, we hypothesized that dietary FO intervention, concurrent with chemotherapy treatment, would maintain adipose tissue morphology, composition and metabolism compared to a reference group in a pre-clinical model. Rats bearing Ward Colon Carcinoma were fed a semi-purified diet with or without fish oil (2.3% w/w) initiated at the same time as chemotherapy. Rats were killed before chemotherapy, after 1-cycle, or 2-cycles of chemotherapy and periuterine adipose tissue was isolated. Healthy rats with no tumor, no chemotherapy, served as a reference group. To investigate fatty acid composition, adipose tissue triglyceride and phospholipid were isolated using Folch immediately followed by thin layer chromatography and gas liquid chromatography. Histological analysis (hematoxylin and eosin) and Real-time PCR (TaqMan) were also performed. Body weight change from baseline (%) nor relative adipose tissue weight (%body weight) were different between groups. Morphological examination revealed smaller adipocytes in rats receiving chemotherapy, either on control or FO diet, compared to the healthy group. The proportion of EPA and DHA in total PLs significantly increased after both 1 and 2 cycles of chemotherapy in FO group, however, the elevation in the proportions of these fatty acids in TGs occurred only after 2 weeks of FO interventions. Observed reduction in mRNA expression of Peroxisome proliferator-activated receptor gamma (PPAR-γ), lipoprotein lipase (LPL), fatty acid synthase (FAS), acetyl coA carboxylase (ACC) and Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1a) that occurred in the control fed rats was prevented by FO intervention. This animal study will verify the beneficial effect of fish oil supplementation on adipose tissue composition and function that could be effectively translated to clinical practice.

Poster Presentations

35. DLX2 homeobox gene regulation of cell fate decisions in the developing brain - relevance to childhood high grade gliomas and gangliogliomas

J. Zagozewski, S. Japoni, Q. Jiang, F. Van Landeghem, and D.D. Eisenstat
Pediatrics, Medical Genetics and Oncology

Introduction: Pediatric high grade gliomas (pHGG) are localized to different neuroanatomic compartments with significant differences in their gene expression profiles. In Histone H3.3 G34V/R mutants more common in cortical pHGG, DLX2 homeobox gene expression is increased with a corresponding decrease in myelin transcription factor (MYT1) expression. DLX genes are necessary for tangential migration and differentiation of inhibitory interneurons during CNS development. Neural progenitors derived from DLX1/DLX2 double knockout (DKO) mice transplanted into a wild-type forebrain differentiate into oligodendrocytes, providing support for the DLX homeobox genes in neuronal-glial cell fate decisions. Methods: Chromatin immunoprecipitation (ChIP) assays using a DLX2 antibody were followed by qPCR. ChIP-reChIP assays were performed using antibodies that recognize specific histone modifications. Electrophoretic mobility shift assays (EMSA) were performed using recombinant DLX2 protein and oligonucleotide probes from ChIP-specified promoter regions. Reporter gene assays provided functional assessment of protein-DNA interactions in vitro. Target gene expression was assessed comparing wild-type (WT) and DLX1/DLX2 DKO tissues. Results: Homeodomain binding sites were localized to the promoters of Olig2, Myt1, Nkx2.2 and others in silico. ChIP assays confirmed promoter occupancy by DLX2. ChIPseq experiments are underway. EMSA studies demonstrated specific DLX2-promoter complexes. Luciferase assays showed repression of Olig2 and Nkx2.2 reporter gene expression, consistent with co-occupancy of H3K27me3 in WT and increased target gene expression in DLX1/DLX2 DKO embryonic tissues. We also demonstrated DLX2 expression in a cohort of gangliogliomas. Conclusions: Our results support a role for DLX transcription factors in controlling neural progenitor specification by activating GABAergic and inhibiting oligodendroglial cell fates through transcriptional repression of a suite of genes required for oligodendrocyte differentiation. Understanding how pHGG and glioneuronal tumors co-opt these neurodevelopmental programs will lead to novel pharmacologic approaches that promote glioma differentiation.

36. RhoGDI2 as a therapeutic target in cancer

A. Sheshachalam, W. Wong, and G. Eitzen
Cell Biology

The small monomeric Rho GTPases are central regulators of cell morphogenesis. They cycle between GDP-bound inactive state and GTP-bound active state which is membrane targeted via c-terminal lipid tail. The GDP-bound state is sequestered in the cytosol by RhoGDIs which bind the lipid tail in a hydrophobic pocket. Overexpression of RhoGDIs increases cytosolic Rho GTPases levels and inhibits their activation. The human genome encodes three RhoGDIs (GDI1, GDI2 and GDI3). Low expression levels of RhoGDI2 have been associated with poor outcome in patients with bladder. Conversely, overexpression of RhoGDI2 suppressed metastasis in experimental models of invasive lung and bladder cancer.Further analysis indicated that RhoGDI2 suppressed metastasis by altering inflammation in the tumor microenvironment. We have examined the binding of RhoGDI2 to the family of Rho GTPase and found that it binds almost exclusively to Rac1. We have also examined the role of RhoGDI2 in immune cells. Mast cells are tissue-resident immune cells that release pro-inflammatory mediators by a process called degranulation (a.k.a. granule exocytosis which store pro-inflammatory mediators). RhoGDI2 expression was reduced in mast cells using shRNA which impaired the degranulation process. As well, a Rac specific GTPases inhibitor inhibited degranulation. Based on our observations, we propose that RhoGDI2 influences inflammation mediated metastasis via regulation of Rac1 GTPase. Hence, RhoGDI2 can be considered as a therapeutic target in cancers.

Poster Presentations

37. Platelet Derived Growth Factor Alpha Drives Aggressive Phenotypes In Metastatic Papillary Thyroid Cancers

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Surgery

Background: Papillary thyroid cancer (PTC), the most common endocrine malignancy, has an impressive propensity for lymphatic metastases that can resist surgical and radioiodide therapy. To expand diagnostic and treatment options for metastatic PTC, molecular techniques are needed to aid the identification of aggressive variants of PTC and to determine the signaling pathways responsible for the metastatic spread. Receptor tyrosine kinases have been extensively studied due to their aberrant activation in many human cancers. Platelet derived growth factor receptor-alpha (PDGFRα), a tyrosine kinase receptor was recently identified by our lab as a specific marker of the metastatic disease. Objectives: Now, we investigate the aggressive phenotypes driven by PDGFRα and the signaling pathways through which they are established. Methods: Using an inducible Tet-on system, PDGFRα expression was induced in a PTC cell line (BCPAP-Teton- PDGFRα). The effect of PDGFRα expression on cell migration and proliferation was assessed using the Boyden Chamber and trypan blue exclusion assays respectively. Cells were also cultured under three-dimensional (3D) conditions to explore spheroid forming abilities and phenotypes. Results: We show in this study that the induction of PDGFRα expression in BCPAP cells increased their migratory abilities. Also, BCPAP-Teton-PDGFRα cells exhibited invasive-like branching phenotypes when cultured under three-dimensional (3D) conditions. This was in sharp contrast to the control cells (BCPAP-Teton-Empty) which formed compact and dense spheroids. PDGFRα expression did not affect the proliferative abilities of the cells. PDGFRα activation provoked downstream up-regulation of the MAPK/ERK, PI3K/Akt and STAT3 pathways. Chemotherapeutic blockade of these pathways using Ly294002, U0126, and Stattic respectively was sufficient to revert the aggressive phenotypes associated with PDGFRα. STAT3 inhibition had the most significant effect. Conclusions: These results strongly suggest that PDGFRα and its associated signaling pathways may be targeted for the effective treatment of metastatic PTCs.

38. The Arg- N-end rule pathway counteracts the pro-apoptotic truncated BAX via protein degradation.

M. Eldeeb and R. Fahlman
Biochemistry

The Arg-N-end rule pathway targets destabilizing N-terminal residue-containing protein substrates for Ubiquitin-dependent proteasomal degradation. It is shown that this pathway plays crucial roles in cardiovascular development, G-protein signalling apoptosis and genomic stability. Arginyl-transferase (ATE1) is a bona fide component of the Arg-N-end rule pathway that mediates posttranslational N-terminal arginylation, the covalent addition of arginine (R) from tRNAArg onto the N-termini of protein substrates. ATE1 knockout is fatal for mice embryos with serious developmental defects in cardiovascular system and angiogenesis. In the course of Arg-N-end rule-mediated protein degradation, ATE1 promotes protein degradation through the addition of N-terminal arginine to target proteins Upon apoptosis in various cancer cell lines, Bax is cleaved by proteases to generate a pro-apoptotic Cleaved fragment of BAX that has a destabilizing N-terminal aspartate. While several studies demonstrated the potent pro-apoptotic activity of Bax, the regulation of stability of this pro-apoptotic fragment has been elusive. Here, we identify that the cleaved pro-apoptotic fragment of Bax is a novel substrate for ATE1.Tellingly; ATE1 mediates the N-terminal arginylation of cleaved Bax which is subsequently targeted for proteasomal degradation by the UBR1 and UBR2 E3 ubiquitin ligases. Notably, we also demonstrate that ATE1-mediated degradation of the cleaved fragment of Bax counters its pro-apoptotic function in cancer cell lines. Taken together, our data support the notion that ATE1 might represent a novel therapeutic target to enhance apoptosis during cancer treatment.

Poster Presentations

39. Development of glycan sequencing and quantification using mass spectrometry for cancer research

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Chemistry

Glycans have crucial roles in a variety of pathophysiological steps in tumour progression. Glycans regulate tumour proliferation and invasion, and cancer cells have long been recognized to have a significant over-expression of sialic acid residues within the glycans found on the cell surface. Methods to identify differences in the structure of glycoproteins are important for understanding of these roles. We have successfully developed methods for both sample preparation, separation and detection using mass spectrometry for glycopeptide site mapping, glycan profiling, sequencing, and fluorescence label approaches for relative quantification. Glycoproteins are enzymatically digested followed by sample clean up. These glycopeptides are than analyzed by nanoLC-ESI-MSMS. Results are processed through database searches using an in house MASCOT server for peptide/protein identification and the GlycoQuest algorithm within a ProteinScape Server (Bruker Daltonics) for glycan/glycopeptide identification. Glycopeptides are incubated with PNGaseF to release the glycans followed by florescent labeling. Samples are purified and subjected to accurate liquid chromatography coupled with fluorescence and mass spectrometry (HPLC-FLD-MS) analysis in order to determine the elemental composition of the glycans. Relative abundances can be obtained based on integration of fluorescence chromatograms. Separation of the fluorescently labeled glycans was developed on both columns, GlycanPac AXH-1 and AXR-1 Columns. We obtained similar separation with standards of labelled glycans from glycoproteins, fetuin and the alpha-1-acid glycoprotein (AGP). These labeled glycans can also be analyzed by nanoLC-ESI-MSMS for sequence information. We will present representative data sets from our studies, and our methods will be of interest for the analysis of glycoprotein and tissue samples.

40. Characterization of cell death induced by the cyanine dye D112: a potentially selective anti-cancer compound

N. Yang, P. Gilman, R. Mirzayans, M. Weinfeld, B. Montpetit, and I. Goping
Biochemistry

Chemotherapeutic drugs that are used in cancer treatments often cause the death of both cancerous and non-cancerous cells. This non-selective toxicity is the root cause of untoward side effects that limits the therapeutic efficacy. In the 1970s, Kodak Laboratories initiated a screen of approximately 7000 dye structural variants for selective toxicity. Among these, D112 was identified as a promising compound with elevated toxicity against a colon cancer cell line in comparison to a non-transformed cell line. Despite these results changing industry priorities led to a halt in further studies on D112. We decided to revive investigations on D112 and have characterized D112-induced cellular toxicity. In the cell lines that we tested, D112 showed increased toxicity toward transformed versus non-transformed cells. We identified that D112 induced cell death through the mitochondrial apoptotic death pathway. To gain mechanistic insights, we examined the effect of D112 using a yeast model system. D112 decreased cellular proliferation and induced yeast cell death. Interestingly, multiple yeast strains that were deficient in respiratory chain function showed resistance to D112 cytotoxicity. All together, our work suggests that D112 may alter mitochondrial electron transport and increase ROS production, which is supported by the finding that D112 localized to mitochondria. Since cancer cells have a greater dependence on anti-oxidant signaling for survival, D112-induced ROS production may in part account for its heightened sensitivity toward transformed cells. Results from this work identify D112 as a potentially interesting molecule warranting further investigation.

Poster Presentations

41. Expression of ESRP1 is a novel indicator of poor prognosis in breast cancer patients

K. Vincent, S. Findlay, and L. Postovit
Oncology

The genetic alterations contributing to breast cancer pathogenesis are incompletely defined, and identifying independent prognostic features from large sample, genome-wide datasets remains a goal of current research. We used transcriptome profiling of 1062 primary breast cancers and 113 associated normal samples from the The Cancer Genome Atlas (TCGA) to find gene expression associated with breast cancer development and progression. This bioinformatics screen revealed that Epithelial Splicing Regulatory Protein 1 (ESRP1) is expressed at significantly higher levels in primary breast cancer samples compared to normal tissue, and its expression is associated with poor prognosis in discovery and validation patient cohorts.Analysis of copy number changes showed that ESRP1 had a gain or amplification in 45 per cent and 16 per cent, respectively, of patient samples; copy number status was also significantly positively associated with ESRP1 expression. Correlation network analysis revealed specific and novel transcript splice variants that are linked with ESRP1 expression and patient survival. This includes two variants of SLK, a serine-threonine kinase, that have opposite associations with patient survival. We are using a CRISPR-mediated gene editing approach to introduce missense mutations into the ESRP1 locus to generate luminal breast cancer cell lines with functional knockouts of ESRP1. We will determine if there is a causal link between ESRP1 and the differential expression of the correlated SLK isoforms. We will also examine if functional knockout of ESRP1 in vitro reduces aggressive phenotypes such as proliferation and invasion. Our study demonstrates that high ESRP1 expression can serve as an independent predictor of survival in breast cancer, and aims to determine if ESRP1 can functionally promote breast cancer progression.

42. Microtubules as biological wires: electrical effects in solution

D. Friesen and J. Tuszynski
Oncology

Microtubules are essential elements of the cytoskeleton of cells and have been implicated in bioelectrical signaling, which may aid in inter- and intra-cellular signaling and tissue organization, which may be lost in cancerous tissue and cells. While microtubules have been found to amplify electrical signals in solution using micropipettes, we investigate more robust techniques to measure electrical signals through microtubules in physiological-like solution in order to characterize more completely the electrical properties of microtubules and eventually microtubule-actin networks. We have created microelectrodes in a flow cell for investigating the effect of microtubules on solution conductivity and electrical amplification from microtubules. Initial results indicate the introduction of microtubules increases the resistance of the solution between microelectrodes, and reproduction of electrical amplification from microtubules in this system has not yet been achieved. Possible causes of microtubules effect on solution conductivity in this system are analyzed, and the biological implications of these results as well as future improvements to this experimental system are discussed.

Poster Presentations

43. Identifying Novel Inhibitors for the XPA-ERCC1 Protein-Protein Interaction

F. Gentile, J. Tuszynski, and K. Barakat
Physics

Many chemotherapy agents target the DNA of cancer cells, forcing them to die. Nevertheless, an active DNA repair pathway in cancer cells removes these drug-mediated lesions and thereby reversing the therapeutic benefits of DNA damaging agents. Tumor cells can therefore survive, grow and proliferate. In this context, DNA repair inhibitors opened a new avenue in combination cancer treatment. The rationale of using these adjuvant therapies is to block the DNA repair mechanisms from removing the chemotherapy-induced DNA damage, hence optimizing the effect and reducing the required dose of the treatment. A profound example is the nucleotide excision repair (NER) pathway, which removes the DNA adducts induced by platinum-based chemotherapy. Part of this pathway is the ERCC1-XPA complex is over expressed in cancer cells and the only known cellular function so far for XPA is to recruit ERCC1 to the damaged point. Recently, we validated the ERCC1-XPA interaction as a promising target to regulate the activity of the NER pathway. Our earlier small molecule hits were able to specifically disrupt this protein-protein interaction and sensitize cancer cells to cisplatin and UV radiation. Here we continued these efforts to identify more selective and potent inhibitors for this interaction. We employed in silico computational drug design methods to: 1) optimize the structures of the previously identified inhibitors; 2) identify novel scaffolds to develop different lead compounds with similar pharmacophore features. The findings described here form a milestone in discovering novel inhibitors for the NER pathway to improve the efficacy of current platinum-based therapy.

44. A New Noscapine Analogue: Synthesis and Biological Evaluation

P. Ghaly, R. El-Magd, C. Churchill, J. Tuszynski*, and F. West*
Chemistry

Noscapine, a phthalide isoquinoline alkaloid, is a natural product that was first isolated and characterized in 1817 by Pierre-Jean Robiquet. Its widely used as a cough suppressant medication due to its low toxicity profile. In 1998, the Joshi group found that noscapine possesses anticancer activity acting on tubulin. In 2011, our group studied the interaction between noscapine and tubulin, and developed a library of computationally designed noscapine analogues that share a common scaffold and are predicted to bind more effectively to tubulin. In our subsequent attempts to synthesize this common scaffold, we came across an interesting compound that showed promising anti-proliferative activity compared to noscapine. We have performed microtubule binding assays and cytotoxicity assays involving SKBR-3 and paclitaxel-resistant SKBR-3 breast cancer cell lines. Unlike noscapine, the new compound causes microtubule destabilization. We also show computational modeling results that indicate its binding pose in the colchicine-binding site.

Poster Presentations

45. Towards development of Thermo/PH responsive lipogels: A novel nanocarrier for tumor targeted drug delivery

N. Ghasemi, M. Vakili, and A. Lavasanifar
Pharmacy

Purpose: The long-term objective of this study is to develop thermo/pH responsive lipogles (stimulus responsive gels entrapped in liposomes) as new nanocarriers for targeted drug delivery in cancer. Methods: Triblock copolymers of poly(α-carboxyl-co-benzyl carboxylate-ε-caprolactone)-b-poly(ethylene glycol)-b-poly(α-carboxyl-co-benzyl carboxylate-ε- caprolactone) (PCBCL-PEG-PCBCL) were prepared through ring opening polymerization of α-benzyl carboxylate-ε-caprolactone (BCL) by PEG (1450 kDa) followed by debenzylation of block copolymer using hydrogen gas. Prepared block copolymers were characterized for their molecular weight and polydispersity by 1H NMR and gel permeation chromatography (GPC), respectively. Rheological methods and differential scanning calorimetry (DSC) were used to measure the gelling temperature of block copolymer solution. The triblock copolymer was labeled with Doxorubicin (DOX) through chemical conjugation of DOX to the free carboxyl group of PCBCL using NHS/DCC chemistry. To prepare

46. Increased ER expression activates a novel subset of E2-regulated genes in ER+ breast cancers

L. Haddon, S. Hu, K. Formenti, R. Chabba, and J. Hugh
Laboratory Medicine and Pathology

Classically, the two estrogen receptor positive (ER+) breast cancer subtypes, luminal A and luminal B, differ in their ER expression and their response to hormone therapy, with luminal B patients having lower ER levels and poor prognosis when treated with Tamoxifen, an anti-estrogen with documented estrogenic properties. In vitro studies have shown that transfecting exogenous ER can reverse the proliferative effect of estradiol (E2) in ER- (MDA-MB-231) and ER+ (MCF-7, luminal B) cell lines. We hypothesized that this effect of E2 in high ER expressing cells must regulate anti-proliferative genes which might be relevant to luminal A high ER expressing breast cancers. To test this hypothesis we generated a high-ER expressing luminal A-like cell line by transducing MDA-MB-231 cells with a doxycycline-inducible lentiviral ER plasmid (231-ER) and compared the mRNA expression of these cells against the luminal B cell line (MCF-7) after 5 days of 10nM E2 exposure using a custom 120-gene panel run on the Nanostring platform. Our mRNA data has shown 13 genes with significant fold-changes in our 231-ERs vs. MCF-7 under E2-treatment. Of these 13 genes, 11 show down-regulation and 2 show up-regulation for the 231-ER cell line. This 13 gene panel is currently being confirmed using qPCR of RNA extracted from MCF-7 cells transduced with (MCF7-ER) or without (MCF7-EM) our ER plasmid and treated with 10nM E2 to ensure our results are not cell line specific. Our preliminary results confirm our hypothesis that increased ER expression has a direct impact on gene regulation in the presence of E2. Investigation of our novel gene panel could give us further insight into the biological differences between ER+ subtypes and has the potential to improve the treatment for ER+ breast cancer patients.

Poster Presentations

47. Imaging glucose and fructose metabolism in breast cancer: A mouse study

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C. Cheeseman, and F. Wuest
Oncology

The role of [18F]FDG-PET in clinical breast cancer (BC) imaging is still limited since up to 50% of BCs do not express high levels of hexose transporter GLUT1. An interesting alternative target may represent fructose transporter GLUT5. It is elevated in several types of cancer, including BC. The goal of the present study was to analyze uptake characteristics of selected fructose and glucose-based PET radiotracers in a murine BC model in vitro and in vivo. Dynamic PET imaging studies confirmed radioactivity uptake but no trapping of fructose-based radiotracers. While 1-[18F]FDF revealed low uptake levels, uptake of 6-[18F]FDF was significantly higher but was followed by slight a washout over 2 h. 6-[18F]FDG reached maximum uptake at 20 min with no further accumulation over time. Clinical glucose radiotracer 2-[18F]FDG showed continuous increase of uptake. In EMT-6 tumors, GLUT5 mRNA expression was about 20,000-fold lower compared to GLUT1. Comparison of GLUT mRNA levels in EMT-6 tumors with mouse muscle revealed, that mouse muscle also shows higher GLUT5, similar GLUT2 and lower GLUT1 mRNA levels (GLUT1 50:1; GLUT2 1:1.6; GLUT5 1:6). Determination of GLUT5 protein levels, however, exhibited higher expression levels in tumor versus muscle tissue. Uptake of fructose-based PET radiotracers cannot simply be correlated with GLUT5 mRNA levels but nicely correspond to GLUT5 protein levels. Taken together, our results point out the importance of detailed biochemical assessment in combination with functional studies to characterize molecular signatures of breast cancer in preclinical studies.

48. Targeting lysyl oxidase for molecular imaging in breast cancer

M. Wuest, M. Kuchar, S. Sharma, S. Richter, I. Hamann,
L. Vos, J. Mackey, F. Wuest, and R. Lser
Oncology

Lysyl oxidase (LOX) and its family members are copper-dependent extracellular matrix enzymes. Compared to normal breast tissue, LOX expression is elevated in breast cancer (BC), where it plays a central role in metastasis. The goal of the present translational research project was to visualize LOX expression in vivo in preclinical models of BC for molecular imaging. Tissue microarray (TMA) analysis revealed that mRNA of all LOX enzymes, except of LOXL4, were upregulated in human BC biopsy samples obtained from 176 BC patients. All three preclinical BC models (EMT6, MCF7 and MDA-MB231) were found to express LOX on protein level. Confocal microscopy and flow cytometry analysis with EMT-6 cells using a LOX-specific antibody and fluorescent-labeled peptide FITC-GGGDPKGGGGG-NH2 showed baseline expression of LOX under normoxia, which increased under hypoxic conditions. Positron emission tomography (PET) using radiolabeled peptide [18F]FBz-GGGDPKGGGGG-NH2 showed initial high tumor uptake after 5 min following continuous washout over 60 min post injection. Specific interaction of the radiopeptide with LOX in vivo was analyzed with the irreversible LOX inhibitor beta-aminopropionitrile (BAPN) in EMT-6 tumors resulting in a 30% reduction of tumor uptake. The present study highlights LOX as a novel and promising molecular target for PET imaging of BC. Moreover, TMA data from BC patient samples further underline LOX as an innovative drug target for BC patient management with metastatic disease.

Poster Presentations

49. Biomaterial that induces localized de-differentiation of breast cancer cells to stem-cell-like state

Y. Hao, J. Wickware, and R. Derda
Chemistry

Breast cancer stem cells (BCSCs) are a small fraction of breast cancer cell populations that have a self-renewing capacity, are resistant to chemotherapeutic drugs, and have the ability to divide asymmetrically, and induce tumor formation. The goal of our project is to identify new biomaterials that can induce non-stem cancer cell (NSCC) reversal to the cancer stem cell (CSC) state. Better understanding of the chemical factors that cause NSCC to CSC conversions could help understand cancer stem cell formation and improve chemotherapeutic treatments. We developed instructive two-dimensional materials covalently modified by peptides to test for induction of localized epithelial-mesenchymal transition (EMT) in breast cancer cell lines, as well as the concomitant NSCC-to-CSC conversion. We found that self-assembled monolayers (SAMs) of the peptide MHRMPSFLPTTL increased the fraction of cells with the BCSC marker profile CD44+/ CD24low- in the luminal breast cancer cell line MCF-7 and basal breast cancer cell line Sum149. Flow cytometry assays indicated that the peptide increased CD44+/CD24-low fraction by decreasing CD24 expression both in MCF-7 and Sum149 cell lines. Mammosphere formation assays showed that MCF-7 cells grown on the peptide for 4 days induced 1.33 times ($p=0.003$) more mammosphere forming capacity in the first generation. mRNA and protein analysis demonstrated that e-Cadherin (an epithelial marker) was significantly reduced in MCF-7 cells grown atop the peptide. This indicates the peptide induces EMT in MCF-7 cells. In summary, we produced a biomaterial that induces EMT and the NSCC-to-CSC transition after 4 days of culture on top of the biomaterial in MCF-7 and Sum149 cells. We anticipate that it will be used for rapid scaled-up culture of CSC for drug testing and diagnostic purposes.

50. Selective Targeting of Human Neuraminidase Enzymes in Cancer Metastasis

M. Howlader and C. Cairo
Chemistry

Human neuraminidase enzymes (hNEU) are a class of enzymes (NEU1, NEU2, NEU3 and NEU4) implicated in several pathologies including cancer and diabetes. Several reports have linked hNEU activity to the regulation of cell migration. The beta-1 integrins, such as VLA-4, are the most likely candidate receptor involved in this process. Using an in vitro cell migration assay, we have investigated the role of these enzymes, as well as the potential of specific inhibitors to interfere in the migration of cancer cells. Four human cancer cell lines were tested with potential inhibitors of the hNEU enzymes including inhibitors previously reported from our group. We have observed that human breast cancer cells (MDA-MB-231) and Prostate Cancer cell lines (PC3) show significant retardation (74% and 60% respectively when compared to controls) of cell migration in our model when treated with a specific inhibitor of NEU3. This cell line was sensitive to 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA), a non-specific inhibitor of hNEU enzymes and Cytochalasin D, a potent inhibitor of actin polymerization. However, selective targeting of NEU3 by inhibitors yielded a substantial reduction over non-specific inhibitors. Targeting NEU4 enzymes by a selective inhibitor resulted in significant reduction of migration compared to nonspecific inhibition with DANA. Inhibition of cancer cell migration using inhibitors of human neuraminidase enzymes has significant potential in the development of new anti-adhesives and adjuvant therapies.

Poster Presentations

51. Encapsulation of an anti-STAT3 agent for enhanced delivery and improved safety

Y. Huang, A. Soleimani, C.S. Wu, Y. Morrissey, A. Lavasanifar, and R. Lai
Laboratory Medicine and Pathology

Signal transducer and activator of transcription 3 (STAT3) is an oncoprotein that is found to be abnormally active in many cancers. The oncogenic effects of STAT3 is exerted mainly by upregulating a series of genes resulting in increased cell proliferation, migration, angiogenesis and reduced apoptosis. The most common STAT3 activation pathway is through upstream phosphorylation by Jak or v-Src, followed by self-dimerizaion, nucleus entry, downstream promoter binding and eventually DNA transactivation. Although Many STAT3 inhibitors targeting the SH2 domain, which is responsible for STAT3 phosphorylation and dimerization have shown extraordinary in vitro tumor-eliminating capacity and specificity, few of them are clinically administered or in clinical trials due to their hydrophobic chemical properties and likelihood to enter normal tissues. The enhanced permeability and retention (EPR) effects suppose that encapsulation of anti-cancer agents in nanoparticles will increase drug accumulation and administration safety. Therefore, here we utilize a synthesized and amphiphilic copolymer to package a STAT3 inhibitor, S3I-1757. We compared the biochemical and biological effects of these packaged S3I-1757 with free S3I-1757 in anaplastic large-cell lymphoma, a cancer model known to have high STAT3 activity. We found that packaged S3I-1757 have comparable STAT3 inhibiting ability, tumor cell toxicity and supreme water solubility and stability. The outcome of this study opens an avenue for small STAT3 inhibitors to medical applications. Moreover, the approach of polymeric packaging can be applied to other highly hydrophobic chemicals for improvement of treatment for other diseases.

52. Matrix metalloproteinase-2 activation in doxorubicin-induced cardiotoxicity alters intracellular Ca2+-signalling

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H. Young, K. Ballanyi, and R. Schulz
Pediatriacs and Pharmacology

Doxorubicin, an anthracycline-class chemotherapeutic agent, is commonly used to treat breast, ovarian and pediatric cancers. However, a dose-limiting cardiotoxicity limits the use of this otherwise effective drug. This cardiotoxicity is incompletely understood, but two key characteristics are elevated oxidative stress and altered intracellular Ca2+-signalling. Oxidative/nitrosative stress can activate matrix metalloproteinase-2 (MMP-2), an intra- and extra-cellular protease that can proteolyze the Ca2+-handling protein calreticulin. MMP-2 is also present in the mitochondria-associated membrane, a key nexus of intracellular Ca2+ signalling. We hypothesized that MMP-2-mediated proteolysis of Ca2+-handling proteins plays a role in the impaired Ca2+ signaling of doxorubicin-induced cardiotoxicity. We found that SERCA, required for Ca2+ re-uptake by the sarcoplasmic reticulum, could by proteolyzed by MMP-2 in vitro, yielding a 50 kDa fragment whose identity was confirmed by mass spectroscopy. This was prevented by the MMP inhibitor GM-6001, and suggests that it could be cleaved by activated MMP-2. To assess the effect of doxorubicin-induced MMP-2 activation on Ca2+-signalling, neonatal rat ventricular myocytes (NRVMs) were treated with 0.5 M doxorubicin for 24 hours, with or without the MMP inhibitors ONO-4817 or ARP-100 (1 M each). Intracellular Ca2+ and mitochondria were visualized in live NRVMs by Fluo-8l and MitoTracker Red, respectively, using confocal microscopy. Both the amplitude and frequency of Ca2+ transients in NRVMs were decreased after doxorubicin treatment. This effect was partly ameliorated by the MMP-2-specific inhibitor ARP-100. In contrast, no changes in SERCA or calreticulin protein levels were observed in cell lysates. Thus, MMP-2 appears to be contributing to the detrimental effects of doxorubicin on cardiomyocyte Ca2+ signaling, but under the conditions tested it may not be acting through SERCA or calreticulin. In conclusion, while the underlying mechanisms have yet to be uncovered, MMP inhibition has the potential to be a viable adjunct therapy in doxorubicin treatment of cancers.

Poster Presentations

53. Treating Bladder Cancer with Oncolytic Vaccinia Virus From Basic Discovery to Clinical Development

K. Potts, C. Irwin, N. Favis, R. Moore, D. Evans, and M. Hitt
Medical Microbiology and Immunology

Introduction: Bladder Cancers (BCa) has a recurrence rate of up to 80% and many patients require multiple treatment strategies that often fail leading to disease progression. In particular, the current standard of care for high-grade disease, Bacillus CalmetteGurin (BCG), fails in around 30% of patients. Oncolytic viruses preferentially replicate in and lyse cancer cells while sparing normal cells. Virus replication requires an abundant supply of dNTPs, but because cellular enzymes that synthesize dNTPs are degraded at the end of Sphase, vaccinia virus (VACV) expresses its own biosynthetic enzymes including both I4L (large, R1) and F4L (small, R2) subunits of the heterodimeric ribonucleotide reductase. We have shown that deleting the F4L gene inhibits virus replication in normal cells while retaining replication proficiency in cancer cells. Results and Methods: We developed preclinical and clinical grade VACVs tagged with the gene encoding mCherry and deletions in F4L, J2R (viral thymidine kinase) or both. Cytotoxicity assays show a high degree of cell killing in infections with VACVs. Highly efficient VACV replication was seen in our BCa cells while limited replication was seen in normal bladder cells or primary fibroblasts. We show that our VACVs selectively replicated in both the orthotopic AY-27 immunocompetent and RT112-luc immunocompromised models causing significant tumor regression or complete ablation with no toxicity. Additionally, immunocompetent rats treated with the VACVs developed a protective anti-tumor immunity that was evident by tumor rejection upon challenge and by in vitro assays. Conclusion: Our data indicate that the VACVs have retained much of their replication proficiency and cytotoxicity despite deletions of critical viral replication genes. If successfully the VACVs could provide an essential line of therapy for patients that do not respond to BCG. This presentation will discuss the current status of our program and our progress towards a clinical trial in bladder cancer.

54. Role of Transcription Factor AP2 in Glioblastoma

S. Jain and R. Godbout
Oncology

Glioblastomas (GBM) are highly invasive brain tumours. Patients diagnosed with GBM have a dismal survival, ranging from 5 months to 2 years. Activator Protein 2 (AP2) is a family of transcription factors (AP2a, b, c, d and e) involved in the regulation of genes responsible for early development, cellular growth, differentiation and apoptosis. Our data show that knockdown of AP2a results in increased cell migration and decreased cell proliferation and cell survival in U251 and T98 GBM cells. Intriguingly, loss of AP2a in the nucleus and increased localization in the cytoplasm have been reported in GBM. Our immunofluorescence and nuclear-cytoplasmic fractionation analyses have revealed that AP2b and AP2c are also located in the cytoplasm of GBM cells. AP2 genes have been shown to be cAMP responsive. cAMP levels in GBM are lower than in normal brain, which may lead to reduced AP2 activity. Phosphodiesterase inhibitors such as Rolipram, which increase intracellular levels of cAMP, have been shown to inhibit GBM cell growth and invasion. In preliminary experiments, we have found that Rolipram increases the nuclear localization of AP2c, suggesting a direct link between increased cAMP levels and the subcellular localization of AP2. To further investigate the role of AP2 in GBM we are using patient-derived tumour neurosphere cultures. Based on our recent data, AP2b is more highly expressed in A4-004 neurospheres compared to adherent cultures derived from the same patient. This suggests that AP2 may regulate stem cell maintenance and differentiation in GBM. Our objective is to further investigate AP2 subcellular localization in GBM cells and determine its effect on gene regulation and growth properties. We will also examine the effect of AP2 on GBM stem cells and resistance to therapy. Determining the role of AP2 subcellular localization on GBM pathogenesis may help develop new forms of therapy for GBM.

Poster Presentations

55. Posttranscriptional Regulation of Wingless in the Drosophila Salivary Gland

A. Jandura and A. Simmonds
Cell Biology

Wnts indirectly activate transcription of genes involved in cell proliferation and differentiation. Extracellular binding of Wnts to receptors prevents formation of the β-catenin degradation complex. Stabilization of cytoplasmic β-catenin allows β-catenin to accumulate in the nucleus and act as a co-transcriptional activator. Wnt1 has been implicated in a number of human cancers, including breast cancer. Drosophila third instar larval salivary glands are used as a model to study breast cancer, as breast and salivary gland tissue are both composed of polarized epithelial cells and share a secretory function. As well, the Wnt1 pathway is conserved in Drosophila; Wingless (Wg) is the Wnt1 Drosophila homolog. wg mRNA is post-transcriptionally regulated through apical subcellular localization due to elements in the 3UTR. Two wg transgenes were constructed that each contain an internal triplicate FLAG tag within the wg ORF, and either possess or lack the 3UTR. These wg transgenes were transfected into S2 cells in order to detect the FLAG tag through IF and Western blotting. Following creation of transgenic wg fly lines, Wg was overexpressed in salivary glands using the UAS Gal4 system. The effect of the wg 3UTR on Wg expression in the salivary gland will be elucidated through future immunofluorescence (IF) and Western blot hybridization assays; immunofluorescence using fixed salivary glands demonstrate the expression pattern, while Western blots show relative Wg levels in transgenic lines. Differences in Wg levels between the two transgenic wg lines would suggest a regulatory function of the 3UTR. Future experiments will include a comparison of RNA detection systems that involve a specific RNA aptamer-protein binding complex such as MS2, Spinach2, Mango and Csy4. Constructs composed of an RNA aptamer and the wg 3UTR will be used to both image and purify wg mRNA.

56. Modelling The PD-L1:B7-1 Protein-Protein Interaction In The Tumor Microenvironment

H. Hasani, M. Ahmed, and K. Barakat
Pharmaceutical Sciences

Programed death ligand-1 (PD-L1) and B7-1(CD-80) are two transmembrane ligands that bind to different receptors of the immune system. PD-L1 delivers an inhibitory signal to T-cells upon binding to PD-1. On the other hand, B7-1 has two opposite roles in T-cells regulation; stimulation through its interaction with CD-28 and inhibition through its interaction with the CTLA-4 receptor. Blocking the interaction of B7 family immune checkpoint proteins with their ligands has emerged as a game changing approach in anticancer therapy. Most interestingly, recent discoveries identified a new role for both B7-1 and PD-L1. The two ligands can directly interact with each other to provide an additional inhibitory signal to T cells, thus deactivating the immune response to Antigen Presenting Cells (APCs). Although this interaction has been confirmed and the binding interface has been partly identified, very little is known about the mechanism and details of their interaction, particularly at the tumor microenvironment. In the tumor context, cancer cells are surrounded by an acidic microenvironment, which can directly affect the nature of the interacting proteins. Here, we continue our efforts in modelling the immune checkpoint proteins and describe, for the first time, and at the atomic level how human B7-1 interacts with human PD-L1 at varying pH. Our methodology combined protein-protein docking algorithms with molecular dynamics (MD) simulations and free binding energy calculation. We hope the findings presented here shed light on how immune checkpoints interact in different physiological conditions.

Poster Presentations

57. Enrichment of Stem Cells Induced by Hypoxia in Breast Cancer

M. Jewer, M. Taylor, C. Hughes, and L. Postovit
Oncology

Hypoxia in the tumor microenvironment promotes cancer growth, metastasis and resistance to treatment. Similarly, Nodal, an embryonic morphogen belonging to the transforming growth factor beta (TGFb) superfamily, has been identified as a factor enhancing metastasis in numerous cancer types. Furthermore, Nodal is a known regulator of pluripotency in Stem Cells and, as we begin to demonstrate here, cancer. Our recent work has shown that, in cancer, Nodal is up-regulated by hypoxia, another regulator of pluripotency. This up regulation occurs while Nodal mRNA levels remain unchanged. Based on this discrepancy we hypothesize that Nodal is regulated by post-transcriptional mechanisms including increased ribosome binding, protein stability and miRNA regulation leading to stem cell like phenotypes. Factors such as mTOR, 4E-BP, and eIF-2α, that control global rates of translation can allow for the translational up-regulation of specific transcripts in response to stress. This translational up-regulation is facilitated by increased ribosome binding to targeted mRNAs. Here, we examine post-transcriptional mechanisms by which hypoxia regulates Nodal by i) measuring the activity of the pathways regulating translation; ii) assessing protein stability; and iii) evaluating a method required to measure the amount of ribosome binding to mRNA termed polysome profiling. Nodals stability increased in low oxygen. Also the mTOR and the eIF2α pathways are active in hypoxia helping to elucidate another potential mechanism of up-regulation. These changes increase EMT and pluripotency increasing multiple cancer phenotypes through the support and maintenance of cancer stem cells.

58. Elimination of Arsenic Species by Single Nucleotide Polymorphic Variants of the Human Multidrug Resistance Protein 2 (MRP2/ABCC2)

G. Kaur and E. Leslie
Laboratory Medicine and Pathology

Arsenic and selenium are toxic compounds, however in vivo exposures to arsenite and selenite result in mutual detoxification. The molecular basis for this can be explained by the biliary excretion of the seleno-bis(S-glutathionyl) arsinium ion [(GS)2AsSe]- by the ATP-binding cassette (ABC) transporter, multidrug resistance protein 2 (MRP2/ABCC2). The ABCC2 gene is highly variable; more than 50 single nucleotide polymorphisms (SNPs) have been identified. Several SNPs have been shown to alter the toxicokinetics of important therapeutic agents. The objective of this study was to determine whether ABCC2 SNPs that result in the amino acid changes, R412G, V417I, S789F, R1150H, R1181L, N1244K, P1291L, V1188E, A1450T, T1477M, C1515Y and C1515Y/V1188E, displayed altered [(GS)2AsSe]- transport activity in comparison to wild-type (WT) MRP2. ABCC2 SNPs were generated using site-directed mutagenesis and expressed in HEK293T cells. Plasma membrane-enriched vesicles were isolated and relative MRP2 levels were determined by western blotting. Cell-surface biotinylation experiments were done to confirm plasma membrane localization of selected variants. Transport activities of WT and variant MRP2 were compared using [(GS)2AsSe]-. All mutants were detected in whole cell lysates except for T1477M. S789F and A1450T were not detected in plasma membrane enriched vesicles. R412G and R1150H displayed lower [(GS)2AsSe]- transport activity compared to WT. The differences in cellular localization of S789F, A1450T and T1477M suggest that these amino acids may contribute to correct folding and trafficking of MRP2. Arsenic exposed individuals with MRP2 SNPs that display reduced transport activity and mislocalization may not benefit from selenium supplementation.

Poster Presentations

59. Cationic Lipopolymer Mediated BCR-ABL Silencing and Implication in Chronic Myeloid Leukemia (CML) Therapy

R. Bahadur KC, B. Thapa, J. Valencia-Serna, and H. Uluda
Chemical and Material Engineering

Chronic myeloid leukemia (CML) therapy based on small molecular drugs, such as tyrosine-kinase inhibitors (TKIs), and stem cell transplantation suffers from acquired TKI resistance and risk of mortality from chronic graft-versus-host disease. siRNA technology could serve as an alternative approach since it works at molecular level to directly interact with abnormal mRNA. In this study, we designed highly hydrophobic cationic lipopolymers by grafting cholesterol (Chol) onto low molecular weight (0.6, 1.2 and 2.0 kDa) polyethylenimines (PEIs) to enable specific siRNA therapy in chronic myeloid leukemia (CML). PEI-Chol readily complexed with siRNAs and formed nano-sized (100 to 200 nm diameter) polyplexes with enhanced ζ-potential (+20 to +35 mV) and ability to protect the loaded siRNA completely in fresh serum. The siRNA delivery to CML (K562) cells was proportional to degree of substitution and, unexpectedly, inversely proportional to molecular size of the polymeric backbone. Chol grafting as little as 1.0 Chol/PEI on PEI0.6 and PEI1.2 enabled silencing of the reporter gene for Green Fluorescent Protein (GFP) as well as the endogenous BCR-Abl oncogene in K562 cells. The PEI-Chol mediated delivery of siRNAs specific for BCR-Abl and KSP genes significantly arrested the growth of K562 cells and induced apoptosis in treated CML cells. The PEIs substituted with aliphatic lipids (lauroyl, palmitic and stearic acid) were not as effective as PEI-Chol polymers in CML cells, while the PEI-Chol polymers were not effective in anchorage-dependent breast cancer cells. Thus, Chol-grafted low molecular weight PEIs appear to be unique siRNA carriers to realize the molecular therapy in CML cells.

60. Structural basis for ASPP family regulation of p53

H. Kim, Y. Zhou, R. Edwards, and J.N. Glover
Biochemistry

Apoptosis stimulating protein of p53, ASPP proteins have long been known to be key regulators of p53, one of the best-known proteins for the high frequency of its mutations in various human cancers. However, the way in which ASPPs control p53 is largely unknown. Our work suggests that the ASPP proteins are potent regulatory partners of human protein phosphatase, PP1. From there, we hypothesize that the ASPP proteins act by regulating the phosphorylation status of p53. We have crystallized and determined a preliminary PP1:iASPP complex structure. The structure reveals an extensive PP1-iASPP contact surface, involving the ANK-SH3 domain of iASPP which docks onto PP1, as well as an extended N-terminal tail in iASPP that binds PP1 and mimics certain interactions observed in other PP1 regulatory factors. We have determined two structures that suggest the complex can exist in open and closed states. In the closed state, the SH3 domain is bound by a PVTPPR motif in the PP1 C-terminal tail. In contrast, dissociation of the C-terminal PP1 tail and opening of the structure could facilitate p53 binding. We suggest that the dynamic opening of the iASPP-PP1 complex and the detachment of the PP1 C-terminal tail may be required for p53 binding and dephosphorylation. Our work provides new insights into the fundamental molecular mechanisms that regulate cancer in human cells. Moreover, the detailed structural information on these complexes revealed by these studies will provide a rational basis for the development of drugs that could be leads for new cancer therapy development.

Poster Presentations

61. Factors Influencing the Utilization of Cancer Screening Services in Indigenous Peoples in the Canadian Arctic: Results of the ACCESS project

F. Kolahdooz, K. Yi, S. Jang, J. McKeen, K. Launier, M. Pakseresht, M. Daemi, and S. Sharma
Medicine

Canadas territories, which are located in the northern parts of North America and are home to large populations of Indigenous peoples, have the highest age-standardized rates of cancer mortality. Low uptake of cancer screening services could contribute to delayed diagnosis, advanced stage of cancer at diagnosis, and less favorable clinical outcomes. Remote Indigenous communities have disproportionately lower utilization rates of some types of cancer screening services. Knowledge of the factors contributing to low uptake rates and the most effective ways to promote uptake is limited. The ACCESS project examined Attitudes towards Cancer in Arctic Indigenous Communities and Examining uptake of Screening Services in 368 Indigenous peoples in two Northwest Territories communities. As a sub-study we present here the results from focus group discussions addressing the following questions: (1) what positive and negative factors do you think influence the utilization of cancer screening services? and (2) what are the ways of promoting the uptake of the services? We conducted four focus groups and two one-on-one semi-structured interviews with local healthcare providers, community stakeholders, and Elders (n=22). We analyzed data using NVivo-10. Perceived positive and negative factors influencing the utilization of cancer screening services were identified. The participants suggested five approaches(a) healthcare providers reaching-out to communities, (b) more collaboration between stakeholders, and (c) sustainable, (d) culturally-relevant, and (e) culturally-acceptable programs and subsequent practical strategies for future policies and actions. Participants also identified (a) experiential knowledge, (b) available resources, (c) increased awareness, and (d) established healthcare systems, as opportunities for promoting the uptake of cancer screening services. According to one participant, They [men] dont go for checkup, unless they are in so much pain. They go to hospital crawling. We have provided empirical knowledge about barriers to and opportunities for improving the uptake of cancer screening services in remote Arctic Indigenous communities. Acknowledgments/Sources of funding: The authors would like to express sincere thanks to all participants, who shared their invaluable insights for this study. We are very grateful to Alberta Innovates Health Solutions for funding this project. We would like to thank the Department of Health and Social Services, Government of the Northwest Territories for supporting this initiative.

62. New fluorescent probes for breast cancer imaging

V. Kondapi, O. Soueidan, C. Cheeseman, and F. West*
Chemistry

GLUTs are membrane proteins associated with cell walls to facilitate hexose transport from extracellular space to intracellular space and vice versa. GLUTs are overexpressed in tumor cells due to high metabolic rates. GLUT1 is overexpressed in almost all tumor cells to facilitate D-glucose transport. As result, tracer development research for cancer imaging mainly focused on developing high affinity ligands for GLUT1 transport protein. While GLUT1 is overexpressed ubiquitously in cancer cells, GLUT5, a D-fructose transporter, is specifically overexpressed in breast cancer cells. Therefore, developing probes that target GLUT5 could potentially improve signal to noise ratio through selective accumulation of tracer in the tumor cells. Thus, our interest is to develop GLUT5 targeting probes for molecular imaging of breast cancer. We performed a systematic study to find a new ligand with high affinity for GLUT5 and one of the potent ligand was used to develop variety of probes for breast cancer imaging. A brief overview of our journey towards the probe development will be presented.

Poster Presentations

63. Small nucleolar RNAs for Breast Cancer Prognosis

P. Krishnan, S. Ghosh, B. Wang, D. Li, J. Mackey, O. Kovalchuk, and S. Damaraju
Laboratory Medicine and Pathology

Introduction: Small nucleolar RNAs (snoRNAs) are small non-coding RNAs that are predominantly involved in biogenesis (rRNA) and maturation of tRNAs and rRNAs. Recently, studies have highlighted their roles in apoptosis, cell proliferation, etc. snoRNAs have shown promise as potential diagnostic/prognostic markers for lung and leukemia cancers. However, their roles in breast cancer (BC) remains less explored compared to mRNA/miRNA signatures. Objectives: (i) To profile and identify differentially expressed (DE) snoRNAs in BC and (ii) To identify snoRNAs associated with clinical outcomes (Overall survival, OS and Recurrence Free Survival, RFS). Methods: Small RNAs were sequenced from 104 BC tissues and 11 normal breast tissues and the generated .bam files were analyzed using Partek Genomics Suite 6.6. Following data filtering and QC, snoRNAs with fold change more than 2.0 and FDR cut-off 0.05 were considered as DE. For the second objective, two approaches were used: case-control (CC) and case-only (CO). For both the approaches, snoRNAs significant in the univariate Cox proportional hazards regression model were used for constructing risk score, which was then adjusted for potential confounders in a multivariate Cox model. Results: In the CC approach, 768 snoRNAs were profiled, 88 were retained after filtering, and 40 were DE. In the CO approach, 763 snoRNAs were profiled, 95 were retained after filtering. In the CC and CO approaches respectively, five and twelve snoRNAs were associated with OS, and four and ten snoRNAs were associated with RFS. Patients belonging to high-risk group were associated with poor outcomes and the risk score was significant after adjusting for confounders. Representative snoRNAs were validated by qRT-PCR. The findings will be validated in external dataset (TCGA). Conclusions: Dysregulation of snoRNAs in breast tumors indicate that these RNAs could potentially contribute to tumorigenesis. This is the first study to delineate the role of snoRNAs in BC prognosis.

64. Germline copy number variations and their role in breast cancer risk

Kumaran, Hubaux, and Damaraju
Laboratory Medicine and Pathology

Background: Breast cancer (BC) is a complex polygenic disease and multiple low penetrant variants play a role in disease etiology. Hence, there is need to identify genetic variants associated with BC, independent of single nucleotide polymorphism based approaches. Copy number variations (CNVs) are an important class of heritable germline polymorphisms; these are large structural variations (more than 1kb in size) encompassing functional genes, regulatory elements, non- coding RNAs. Recent studies have reported role of germline CNVs as heritable determinants in complex polygenic diseases. Hypothesis: CNVs can explain a proportion of the heritability in BC risk. Materials and Methods: A two-stage approach was adopted to identify robust signatures associated with BC in Caucasian subjects. Discovery cohort: 422 BC cases and 348 Controls, cohorts recruited within Alberta Canada. Validation cohort: 495 BC cases, from The Cancer Genome Atlas project (TCGA) and 1347 Controls Welcome Trust Case Control Consortium (WTCCC), data was accessed from public repositories. Germline CNV profiles were generated on Affymetrix Human SNP 6.0 arrays. Quality control steps were implemented and ancestry verified using Eigenstrat algorithm. CNVs were detected by Genomic segmentation algorithm implemented in Partek Genomic Suite 6.6 using HapMap data as reference baseline and adopting softwares default parameters. The CNVs associated with BC were tested using Pearsons chi-square test and corrected for false discovery rate (FDR, q-value) of less than0.05 was considered significant. Results: We identified significant CN gains (n=151) and CN losses (n=257) that are concordant between the stages showing similar genotype frequencies and effect sizes. Signatures annotated using CNV annotator databases mapped to the gene regions, long non-coding RNAs, piRNAs, snoRNAs. These findings imply that CNVs play a functional role and can potentially confer BC risk. Conclusion: The replication of findings from validation cohort reflects the robustness of the identified signatures. These signatures will be validated using qRT-PCR.

Poster Presentations

65. Hypoxia and Nodal: Epigenetic Modulators in the Development of Cancer Stem Cells

L. Lee and L. Postovit
Oncology

Nodal, an embryonic morphogen, and hypoxia promote pluripotency in human Embryonic Stem Cells (hESCs), but also support cancer progression when signalling pathways are dysregulated. Both of these factors have been shown to epigenetically alter gene expression, and are crucial in the development of Cancer Stem Cells (CSCs). CSCs are a subpopulation of cancer cells characterized by plasticity and self-renewal, which afford CSCs with the ability to metastasize and resist therapies, leading to reduced survival in patients. Here we propose to address how the epigenome of CSCs respond to micro-environmental factors such as hypoxia and Nodal by examining the alterations in histone modifications in concert with the resulting transcriptional response. Cell lines that up-regulate stem cell sustaining genes such as Nodal in response to hypoxia will be used, namely H9 hESC and breast cancer cell lines, SUM149, T47D, and MCF7. To determine epigenetic changes, Chromatin Immuno-Precipitation (ChIP) with high throughput sequencing (ChIP-seq) will be conducted using antibodies to repressive (H3K27Me3) and active (H3K4Me3) histone marks, which were chosen for their association with hypoxia, Nodal, and regulation of the stem cell phenotype. RNA sequencing will be performed to match gene expression changes and PCR will be incorporated to validate ChIP-seq and RNA-seq results. Elucidating the role of histone modifications in the transcriptional response to hypoxia and Nodal, as well as the role of Nodal in hypoxia associated alterations, will better our understanding of how the microenvironment regulates CSCs and ESCs, leading to the discovery of potential therapeutic targets.

66. Selective inhibitor of UBR box as standing blockage of Arg/N-end Rule Pathway in living cells

L. Leitao, M. Eldeeb, and R. Fahlman
Biochemistry

The ubiquitinproteasome system (UPS) is a highly important regulatory mechanism of protein catabolism and homeostasis in cells. This proteolytic system target short-lived substrates depending on their N-termini targeting them to degradation. The recognition of destabilizing N-terminal amino acid residues of target protein substrates is mediated by the redundant UBR1/UBR2 E3 ubiquitin ligases that further moderate the substrate ubiquitination and target them for proteasomal degradation. Both UBR1/UBR2 E3 ligases have two crucial recognition domains, the UBR box domain and the N-domain, which function independently as binding motifs for type I (positively charged basic amino acids as Arg and Lys) and type II (bulky hydrophobic residues as Trp and Tyr) N-terminal destabilizing amino acids, respectively. Recent reports revealed that several pro-apoptotic protein fragments generated by proteolysis by caspases and calpains have type-I basic N-terminal destabilizing residues. These truncated C-terminal protein fragments are selectively degraded via the N-end rule pathway through the action of UBR box domain of the UBR1/UBR2 ligases. Consequently, these ligases mitigate the pro-apoptotic signalling cascade and enhance resistance of cells to apoptosis-inducing agents. The development of specific inhibitor of UBR box domain of UBR1/UBR2 would inhibit degradation of several pro-apoptotic proteins and as a result would sensitize cells to chemotherapeutics or potentially radiation treatment. In order to investigate the inhibition of this pathways by small molecule inhibitors, protein half-life assays are done with a cleaved form of the Lyn tyrosine kinase as a reporter protein. Lyn is cleaved in its N-terminal by caspase-3 and selectively targeted for degradation by the N-end rule pathway. The inhibition of degradation of such fragments would sensitizes CML cell lines to apoptosis-inducing agents. Here we show our studies on a novel inhibitor of UBR box domain of UBR1/UBR2, using Lyn reporter protein fragments with different N-termini as model substrates, in living cells.

Poster Presentations

67. Inhibition of Wee1 with MK-1775 prevents mitotic exit and promotes mitotic catastrophe

C. Lewis, Z. Gin, D. McDonald, W. Wei, and G. Chan
Oncology

Wee1 kinase is an important cell-cycle regulator that has two major roles: First, to prevent premature entry into mitosis or mitosis with damaged DNA, Wee1 inhibits the mitotic promoting factor (Cdk1/cyclin B1) by adding a phosphate onto tyrosine 15. Second, Wee1 kinase has been recently shown to be required for mitotic exit. We have tested the inhibition and knockdown of Wee1 through use of a small molecule MK-1775 (an anticancer drug in phase I/II clinical trials) and specific siRNAs respectively. We found that G1/S synchronized cells treated with MK-1775 or siWee1 prematurely enter mitosis and display the Mitosis with Unreplicated Genome (MUG) phenotype. These cells also exhibited a prolonged mitotic arrest and dye by mitotic catastrophe (a common mode of cell death that has a poorly defined molecular mechanism). We also treated cells synchronized at prometaphase with MK-1775 and found that Wee1 inhibition prolongs the metaphase-anaphase transition 10-12 fold compared to DMSO controls. Moreover, MK-1775 treated cells failed to phosphorylate Cdk1 on tyrosine 15 and degrade cyclin B1. Together our data suggests that failure to exit mitosis normally promotes mitotic catastrophe. We hypothesize that targeting other proteins involved in mitotic exit will enhance the efficacy of MK-1775 in inducing mitotic catastrophe. In support of this, we have found that HeLa (cervical) and MDA-MB-231 (breast) cells co-treated with MK-1775 and the anti-microtubule drug Taxol decreases cell viability by 50% compared to either Taxol or MK-1775 alone. We are now screening a siRNA library of human genes with MK-1775 as a mean of identifying other genes that promote mitotic catastrophe. Our screen will have the clinical benefit of indentifying tumour suppressors that when silenced or mutated enhance the efficacy of MK-1775 for mono- or combination therapies. Furthermore, our screen will help to define molecular pathways of mitotic catastrophe.

68. Ovarian Cancer Biomarker CA125 Expression is Attenuated in in vitro Culture

J. Liu, H. Steed, Y. Fu, C. Lee, B. Meng, G. Zhang,
K. Vincent, D. Dieters-Castator, D. Pink, J. Lewis, and L. Postovit
Oncology

CA125, a 22,152 amino acid protein encoded by MUC16, is a glycoprotein that is used as a biomarker for ovarian cancer. Approximately 80% of ovarian cancer patients present with high levels of serum CA125. Overexpression of this biomarker in ovarian cancer indicates its possible role in cancer pathogenesis; however studies related to CA125 function in cancer development are quite limited. During our research on biomarkers we found that only one out of seven ovarian cancer cell lines (NIH:OVCAR3) expresses CA125. Accordingly, we hypothesized that CA125 may be lost during adaptation to cell culture conditions. In support of this concept, analysis of publicly available data sets, including The Cancer Genome Atlas (TCGA) revealed that while the majority of primary ovarian cancer sample express high levels of MUC16, only three out of fifteen ovarian cancer cell lines express appreciable amounts. We confirmed this result by demonstrating that CA125 could not be detected in the cell lysates, microvesicles or supernatants of six ovarian cancer cell lines (A2780s, A2780cp, SK-OV3, OV-90, ES-2 and OVCA429). It could, however, be detected in all fractions from OVCAR3 cells. Four of ten cultured ascites cells from ovarian cancer patients, whose clinical CA125 levels were above 35 kU/L, showed detectable CA125 by Western blot. In contrast, CA125 could be in detected in almost all lysates, microevesicles and supernatants derived from ascites that had never been cultured. In order to determine if CA125 was indeed lost over time, we compared CA125 protein and mRNA levels in primary ovarian cancer cells before and after successive passages. We determined that both transcript and protein were lost as early as one passage in culture. Notably, we insured that we were culturing ovarian cancer cells (and not contaminating stromal cells) with sequencing for mutations as well as Western blotting for the epithelial ovarian cancer markers Cytokeratin 7 (CK7) Cytokeratin 20 (CK20) and Estrogen Receptor (ER). Taken together, our results indicate that ovarian cancer cells rapidly lose the expression of CA125 as they are adapted to in vitro culture conditions. Hence, caution should be used when using cell lines for biomarker discovery.

Poster Presentations

69. S- and N-palmitoylation are novel post-translational modifications of Glutathione transferase P1 (GSTP1).

V. Marensi, M. Yap, L. Berthiaume, and E. Leslie
Physiology

Glutathione transferase P1 (GSTP1) protects cells from carcinogens by catalyzing their conjugation with the tripeptide glutathione (γ-Glu-Cys-Gly). GSTP1 is also involved in cell signalling, proliferation and apoptosis. Overexpression of GSTP1 in tumours and single nucleotide polymorphic variants are associated with anti-cancer drug resistance and poor prognosis. In contrast, inactivation of GSTP1 due to epigenetic promoter silencing increased susceptibility to certain cancer types, prostate cancer being the best studied example. GSTP1 is classically described as a cytosolic enzyme; however, we have reported that it is strongly associated with the plasma membrane and the strength is comparable with an integral membrane protein. We hypothesize that the addition of a hydrophobic component is required to allow its strong interaction with membranes. Palmitoylation is the reversible post-translational addition of a 16-C saturated fatty acid to proteins, most commonly on Cys residues through a thioester bond. We found that GSTP1 is modified by palmitate. However, Cys-less (Cys to Ser or Cys to Ala) mutants expressed in MCF7 cells surprisingly retained palmitoylation. In addition, treatment of palmitoylated GSTP1 with NaOH, which cleaves thioester bonds, did not remove palmitate. These data together suggested that GSTP1 is modified by palmitate at multiple sites, including at least one non-Cys residue. We also demonstrated that GSTP1 can be non-catalytically palmitoylated (or autoplamitoylated) in vitro using purified GSTP1. Peptide sequencing by ESI-MS/MS of the GSTP1 autopalmitoylated in vitro revealed that Cys48 and Cys102 undergo S-palmitoylation while surprisingly Lys103 undergoes N-palmitoylation. N-palmitoylation is a rare and novel type of post-translational modification that occurs via a very stable amide bond and provides an explanation for the resistance of GSTP1 palmitoylation to NaOH treatment. Future work will validate the extent of palmitoylation and identify palmitoylated residues of GSTP1 isolated from the human breast cancer cell line MCF7 using ESI-MS/MS peptide analysis as above.

70. Stratifying advanced cancer patients according to performance status, weight loss, and systemic inflammation contributes to overall survival discrimination

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A. Voss, C. Deans, K. Fearon, F. Bozzetti, and V. Baracos
Agricultural, Food and Nutritional Science

Background: ECOG Performance status PS describes patients ability to function in relation to their daily life, and is a standard criterion for treatment eligibility and entry into clinical trials. Other clinical assessments may add to the utility of PS. Purpose: To further refine the survival discrimination of patients with good or poor PS using two additional variables: BMI-WL Grades Martin L et al 2015, J Clin Oncol: a classification for cancer-associated WL linked to increased mortality associated with decreasing BMI and increasing WL Grade 0high BMIno WL; Grade 4low BMIhigh WL. Glasgow Prognostic Score GPS: grades inflammation by combining abnormal levels of albumin andor C-reactive protein GPS 0CRPle10 mgLAlbumin ge35gL; GPS 2CRPgt10 mgLAlbumin lt35 gL Methods: The prognostic impact of ECOG PS, BMI-WL grades, and GPS was evaluated in an international database of advanced cancer patients gastrointestinal, breast, respiratory, headandneck, genitourinary. Variables were entered into a multivariable analysis controlled for age, sex, and cancer site. Results: In the sample N2,656 median overall survival was 7.6 months 95 CI 7.1-8.1. BMI-WL grades, GPS, and ECOG PS independently predicted overall survival plt0.001. Patients with good PS ECOG PS 0-1 had incremental reductions in median survival with increasing WL and inflammation, spanning 23.5 months BMI-WL grade0-1GPS0 to 3.6 months BMI-WL grade4GPS2; Plt0.05. Similar results were observed for ECOG PS 2 median survival from 13.5 to 2.8 months, Plt0.05, and for patients with poor PS ECOG PS 3-4 median survival from 12.2 to 2.0 months, Plt0.05, with increasing inflammation and WL. Conclusion: BMI-WL grades and GPS may be useful in clinical decision-making. The simultaneous presence of inflammation and WL is associated with high mortality, even in patients of good PS. Likewise patients of poor PS but lacking inflammation or WL have extended survival times that exceed some patients with apparently good PS.

Poster Presentations

71. Use of Droplet Digital PCR for Ultrasensitive Gene Expression Profiling and Mutational Analysis of Thyroid FNA Biopsies

A. Matkin, M. Kostiuk, D. OConnell, H. Seikaly, D. Cote, J. Harris, and V. Biron
Surgery

Fine needle aspiration biopsies (FNABs) are currently the gold standard test for diagnosis of thyroid nodules. Unfortunately, up to 20-25% of cytology reports of this nature are classified as indeterminate resulting in repeat testing and, occasionally, precautionary hemi or total thyroidectomy. Advancements in molecular diagnostic technology and increased understanding of the molecular mechanisms contributing to thyroid cancer can increase the ability to accurately detect and treat thyroid cancers from early stages. We propose the use of droplet digital PCR (ddPCR) to accurately identify pathogenic mutations or abnormal gene expression levels obtained from FNABs. Ultrasound-guided FNABs were collected from patients with thyroid nodules and ddPCR was performed using up to 2 ng of cDNA. Gene expression levels were measured relative to internal control EEF for PTEN, PIK3CA, MET, CCND1, MKI67, TSHR, LGALS3, EGFR, and p53. Mutational analysis was performed with comparison to wild type control for HRAS pG12V, HRAS Q61R, HRAS Q61K, NRAS Q61R, NRAS Q61K and BRAF V600E. Genetic profiling results were compared to standard histopathology from FNAB and final surgical pathology for patients who later received hemi/total thyroidectomy. FNAB samples from 69 patients were collected for ddPCR analysis. Patients at presentation were 7:1 female gender with a mean age of 53 years (range 17-85). Compared to other methods of biomarker analysis using FNAB samples, mutational and gene expression data was reliably obtained with small amounts of nucleic acid. Mutations were identified in a number of FNAB specimen. Gene expression profiles demonstrated a broad range of expression for all genes tested. The detection of molecular biomarkers of thyroid cancer through the use of ddPCR is a process that has the potential to become a new diagnostic tool. With this method, we are able to detect specific mutations in thyroid nodules the same day the sample is obtained from the patient.

72. Synthesis of metabolic radiotracer [18F]-fluoro-beta-hydroxybutyrate

S. Mattingly, J. Bailey, E. Fine, F. Wuest, and R. Schirrmacher
Oncology

Positron emission tomography (PET) is a powerful tool in oncology for cancer diagnosis, staging, and monitoring. Currently, the workhorse of that technology is a glucose analogue labeled with the positron emitter fluorine-18, [18F]-fluorodeoxyglucose (FDG). Here, we present our progress toward the synthesis of a new metabolic radiotracer, [18F]-beta-hydroxybutyrate ([18F]-fluoro-BHB). This new tracer is designed to serve as a mimic for the ketone body metabolite beta-hydroxybutyrate (BHB), an important energy source and signaling molecule. In doing so, we will be expanding on work that has been done using a C-11 analog. [11C]-BHB, prepared by other investigators, has been successfully applied to brain imaging in humans; however, the use of the tracer has been limited, probably due to a challenging synthesis and the short-lived radioactive half-life of the radioisotope 11C (20 minutes). [18F]-fluoro-BHB will likely have several practical advantages over [11C]-BHB; the radiochemistry will be more straightforward, making it feasible for a larger number of investigators, the resolution of the PET images will be superior as a result of the lower positron energy of 18F vs 11C, and the longer half-life of 18F (110 minutes) will make it more suitable for clinical applications. In this presentation we detail the evolution of our precursor design and the synthesis of epoxide and cyclic sulfate precursors to [18F]-fluoro-BHB. It is our hope that the PET imaging probe [18F]-fluoro-BHB may be able to serve as a complementary technology to FDG PET by overcoming some of the limitations of FDG for visualization of glycolytic tissues. The monocarboxylate transporters that facilitate cellular entry of circulating BHB have been found to be overexpressed in several cancers including high-grade gliomas and colorectal cancer; therefore there is a potential for [18F]-fluoro-BHB to become an important addition to the existing arsenal of PET radiotracers.

Poster Presentations

73. Regulation of the Bmi1 and Ret proto-oncogenes by the DLX2 transcription factor in the developing gastro-intestinal tract

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Medical Genetics

Introduction: Colorectal cancer is responsible for the second most cancer related deaths. Mutations in oncogenes responsible for regulating cellular proliferation in the GI tract account for increased susceptibility to this cancer type. Within the intestinal crypts there is a stable, non-dividing stem cell group marked by the oncogene BMI1. The role of these cells is to maintain and support the epithelial cell lining of the intestine, which cannot replace itself mitotically. Unpublished work from the Eisenstat lab demonstrates co-expression of BMI1 and the homeobox transcription factor DLX2 in intestinal crypts. Additional unpublished data support a regulatory role of DLX2 over the Ret proto-oncogene. Ret is responsible for enteric nervous system development. Over-expression of Ret induces cancers associated with Multiple Endocrine Neoplasia, and loss-of-function leads to Hirschsprungs Disease, characterized by defects in intestinal innervation. We investigated the potential for a regulatory effect of DLX2 on both Bmi1 and Ret, with the hypotheses that DLX2 suppresses Bmi1 expression and promotes Ret expression and these interactions are due to direct binding of the targets promoters by DLX2 during intestinal and ENS development. Methods: We investigated interactions between DLX2 and target promoters in vivo through Chromatin Immunoprecipitation (ChIP) using our high-affinity DLX2 antibody. Electrophoretic mobility shift assays (EMSAs) and with Site Directed Mutagenesis of DLX2 binding sites are used to determine the direct binding of the Bmi1 or Ret promoters by recombinant DLX2 in vitro. Reporter gene assays are being used to determine the effect that DLX2 has on Bmi1 Ret expression in vitro. Ongoing immunohistochemistry and qRT-PCR assays using Dlx1/Dlx2 double knockout (DKO) mouse-derived tissues are used to determine the role of DLX2 in Ret and Bmi1 expression in vivo. Results: DLX2 interacts with both Bmi1 and Ret promoter regions of interest in vivo. EMSA results demonstrate specific binding of DLX2 to the Bmi1 and Ret promoters in vitro. Conclusion: ChIP results confirm DLX2 occupancy of the Bmi1 and Ret promoters while EMSAs demonstrate direct binding of DLX2 to the promoters in vitro. Future studies include in vivo gene expression assays comparing wild type expression in the Dlx1/Dlx2 double knockout mouse to the wild type will confirm the biological relevance of the in vitro results

74. Targeting Integrin Beta-1 (CD29) to reduce the attachment of Breast Cancer Cells to Bone

D. Sundaram, C. Kucharski, and H. Uludag
Pharmacy and Pharmaceutical Sciences

Purpose: The attachment of breast cancer cells with the aid of integrins to extracellular (ECM) proteins such as fibronectin and vitronectin present on bone marrow environment plays a critical role in the metastasis of tumor cells to bone. This attachment acts as a primary site of interaction between cell populations in addition to ECM binding, and allows the tumors cells to remain dormant in bone for a longer period of time and proliferate. Blocking this primary site of attachment might be beneficial in reducing metastasis, which could be achieved by silencing the cell surface integrins present on cancer cells. In this study, siRNA targeting integrin beta-1 (CD29) was delivered using modified polyethylenimine polymers, with the purpose of reducing breast cancer cells attachment to ECM and bone cells. Methods: Integrin beta-1 silencing experiments were carried out in MDA MB-231 breast cancer cells through immunostaining, qPCR, fibronectin and human bone marrow stromal cell (hBMSC) adhesion assays and cell viability (MTT) assay. Results: Three in-house designed polymers 1.2PEI-taLA6, 1.2PEI-Lau8 and 1.2PEI-LA6 displayed effective silencing at low siRNA concentration (40 nM), as determined by CD29 immunostaining, with 1.2PEI-LA6 exhibiting best silencing. The surface level of integrin beta-1 were reduced until day 6 after treatment, whereas the mRNA levels remained silenced until day 9. The functionality of this silencing was assessed by studying its ability to attach to fibronectin and human bone marrow stromal cells as integrin beta-1 is a primary receptor for fibronectin. In both these assays, significant reduction in the cell attachment was observed in CD29 siRNA treated cells. Conclusions: The mRNA levels of integrin beta-1 gene at three different time points exhibited significant silencing. Both the fibronectin binding and hBMSC adhesion assay revealed that this amount of integrin silencing at the cell surface was adequate to reduce its binding ability thereby revealing the functional benefit of integrin beta-1 reduction in breast cancer cells. Silencing integrin beta-1 decreased the cell number which could be the result of apoptosis or declined cell proliferation and identifying additional targets to reduce the attachment of breast cancer cells might have better effect on metastasis. Additional studies in animal models will be needed to confirm if metastasis of breast cancer cells will be reduced after integrin beta-1 reduction.

Poster Presentations

75. Epigenetic modification of an endothelial specific gene, Von Willebrand Factor (VWF) potentially increase cancer cells extravasation and metastasis

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D. Eisenstat, C. Sergi, J. Lewis, and N. Jahroudi
Medicine

VWF is an adhesive procoagulant protein that is exclusively expressed in endothelial cells (EC) and megakaryocytes. It is considered as EC marker and is key initiator of blood clotting cascade and thrombus formation. Increased plasma levels of VWF and alterations in coagulation system in cancer patients with metastasis are reported. We hypothesised that a subpopulation of some cancer cells of non-endothelial origin may acquire VWF expression and as a result develop enhanced extravasation and metastatic potential. RT-PCR, western blot and Immunofluorescent (IF) analyses showed significant levels of VWF expression in glioma (U251, M049) and osteosarcoma (Saos2) cell lines. Chromatin immunoprecipitation assays demonstrated similar pattern of epigenetic modifications, including methylation pattern and histone modification, as well as transcription factors binding to the VWF promoter in VWF expressing cancer cells as observed in EC. In vitro cell-cell interaction analyses showed that cancer cell lines expressing VWF, exhibit increased adhesion to platelets and an EC monolayer under sheer stress. Invasion assay showed an increased migration ability of cancer cells expressing VWF and suggested that VWF may help cancer cells passage through EC junctions. IF analyses of human glioma and osteosarcoma tumor samples, demonstrated VWF expression in some cells of non-endothelial origin in the tumor region, highly suggestive of VWF expression in a subset of cancer cells. Chick Chorioallantoic Membrane assays showed increased extravasation and metastatic activity for VWF-expressing cancer cells compared to cancer cells in which VWF expression was knocked down. Subpopulation of cancer cells of non-endothelial origin acquire denovo expression of VWF as a result of epigenetic modification of the VWF promoter, potentially enhancing their metastatic ability.

76. Immune Consequences of Genetic Instability in Colorectal Cancer

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Oncology

Colorectal Cancer (CRC) is increasing due to the prevalence of unhealthy lifestyles in developed countries. A complication of CRC is the tolerogenic mucosal immune system, which hinders recognition of tumour specific antigens. Approximately 80% of CRCs are chromosomal unstable (CIN). This is associated with a poor immune response, making it the most deadly form of CRC. In comparison, microsatellite instable (MSI) CRC only constitutes 15% of all cases, but its high immune response results in a more positive prognosis. Using CRISPR technology, we have knocked out (KO) genes specifically mutated in MSI and CIN CRCs, as well as DNA repair genes mutated in both. We then transfected our CRISPR plasmids into both a CIN CRC (MC38) and a WT small intestinal (MODEK) mouse cell line. Gene KOs associated with numerous small mutations are expected to have an immune response similar to MSI CRC, whereas KOs associated with limited large mutations are expected to have a response similar to CIN CRC. We are co-culturing immune cells with our cell lines to evaluate the immune cell receptors using flow cytometry and the cytokine release using ELISA. For an in vivo model, we have created organoids from intestinal stem cells that we will transfect with GFP-CRISPR plasmids before injecting them into the colon of C57BL/6 mice. Tumour growth in these mouse models will be monitored using both MRI and colonoscopy. In the future we will evaluate the effect of various bacterial metabolisms on tumourigenesis, as well as how various diets affect the microbial contribution to CRC. We plan to use our research to create adjuvant therapies that improve the response of CIN CRCs to available immunotherapies, as well as therapies that use bacterial metabolites to target tumour cells.

Poster Presentations

77. Cellular Localization of the Cleaved Lyn Kinase and Drug Resistance

P. Nagar and R. Fahlman.
Biochemistry

Lyn is a member of the Src family of tyrosine kinases (SFKs). They are key players in a diverse array of functions in cells including apoptosis [1] and its upregulation has be implicated to be one of the mechanisms in the development of Gleevac resistance in Chronic Myeloid Leukemia . The full-length protein is associated with the plasma membranes a result of N-terminal myristoylation and palmitoylation of cysteine at position 3 (C3) [2]. During apoptosis Lyn is cleaved, 18 residues downstream of its N terminus. This means that the fatty chain modified residues that anchor the protein to the membrane are removed, enabling the protein to localize in cytoplasm instead of the membrane. [6-8]. The over expression of this cleaved form the Lyn kinase renders the cells more resistant to Gleevac as determined in the CML derived cell linewn in the chronic myeloid leukemia or CML cell line K562 [3-5]. Studies have shown the cleaved protein to be more drug resistant than the membrane bound full length protein [6]. Thus, we aim to focus our investigation in trying to decipher the role of the membrane localization of Lyn Kinase on its downstream cleavage and eventually its role in aiding cancer resistance to treatment.

78. Understanding the role of microRNAs in human Cancer Cachexia

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Laboratory Medicine and Pathology

Background: Cancer cachexia (CC) is a paraneoplastic syndrome characterized by severe depletion of skeletal muscle with/without fat loss. Although many genes have been identified to play a role in CC, impact of finer gene regulatory mechanisms on CC remains elusive. microRNAs (miRNAs) are small non-coding RNAs (18-22 nucleotides) that are considered as global regulators of gene (mRNA) expression. miRNAs have been implicated in muscle wasting conditions such as Duchene muscular dystrophy. However, a comprehensive understanding on the role of miRNAs in CC is still emerging. Aims: i) To profile and identify differentially expressed (DE) miRNAs from skeletal muscle biopsies; and (ii) to identify putative targets for miRNAs using TargetScan and validate using in-house muscle mRNA dataset. Methods: 43 cancer patients were stratified into 19 cases with weight loss more than equal to 5% and 24 controls that were weight stable in the preceding 6 months. RNA isolated from muscle biopsies were sequenced using Illumina MiSeq platform. miRNAs and mRNAs exhibiting a fold change of more than 1.4 and p less than 0.05 were considered as DE. Pathway analysis was done using Ingenuity pathway analysis (IPA). Results: A total of 781 miRNAs were expressed and 82 miRNAs with more than 5 read counts in 80% of samples were further interrogated. Seven DE miRNAs (up-regulated) were identified (miR-3184-3p, miR-1296-5p, let-7d-3p, miR-532-3p, miR-193b-5p, miR-423-5p and miR-345-5p). Validation of let-7d-3p using qRT-PCR showed similar relative expressions as in sequencing experiments. In-house muscle transcriptome dataset identified 212 mRNA targets and 48 pathways for 7 DE miRNAs. Identified pathways were found to play a role in myogenesis, adipogenesis, inflammation, all of which contribute to CC pathophysiology. Further replication in independent datasets is underway. miRNAs identified in this study have not been studied in the context of CC and may be of value in developing targeted therapeutics.

Poster Presentations

79. Synergism between Doxorubicin and Docosahexaenoic acid increases apoptosis and causes cell cycle arrest in MDA-MB-231 breast cancer cells

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Agricultural, Food and Nutritional Science

Long chain n-3 polyunsaturated fatty acids have been shown to reduce viability of breast cancer cells in vitro and in vivo although the cellular mechanisms by which this occurs have not been clearly elucidated. Our lab group has previously shown that pre-treating MDA-MB-231 breast cancer cells with docosahexaenoic acid (DHA) prior to doxorubicin (DOX) treatment, reduces cellular viability in vitro. In the current study we sought to 1) verify these findings in vivo and 2) explore the effect of DHA treatment prior to DOX on gene expression in MDA-MB-231 cells. Nu/nu mice (6 wk old) were injected subcutaneously with MDA-MB-231 cells (2x106 cells) and fed control diet ad libitum for four weeks. Tumour-bearing mice were then randomized to a diet (control or DHA 5% w/w, n=4/group) and IP injected twice weekly with DOX (5mg/kg) for 5 weeks. Preliminary analysis of extracted tumours found DHA+ DOX tumours to be smaller than the control +DOX tumours (0.75 0.46 grams versus 2.63 0.46 grams p0.03, n=4). In the second experiment in vitro cells were treated with DHA (60 M) in control medium (containing 40 μM oleic acid / 40 μM linoleic acid (OALA)) or control media alone for 48h and then treated for 24h with DOX (4.1 x10-7 M). RNA was extracted for microarray analysis (Affymetrix GeneChip Human Gene 2.0). A selection criterion of p0.05 and fold change 1.5 was set to define up or down regulated genes compared to cells incubated only with the control media (using Ingenuity Pathway Analysis software). Apoptosis and Cell Cycle were identified through microarray analysis as 2 key canonical pathways significantly changed. Multiple genes were down-regulated (including Cyclin B1, Wee1, and cdc25C) or up-regulated (including caspase 10 and BID) after combination of DHA+DOX compared to cells treated with DHA or DOX alone. Subsequent protein analysis confirmed the changes in gene expression. Our results confirm that feeding a diet containing DHA treatment facilitates the effect of DOX on tumour growth and that this may be occurring by amplifying the effect on genes for proteins that regulate cell cycle and apoptosis.

80. A retrospective longitudinal cohort study of micronutrient intake and dietary changes of patients with head and neck cancer

S. Nejatinamini, C. Kubrak, S. Ghosh, W.V Wismer, and V.C. Mazurak
Agricultural, Food and Nutritional Science

Background: Micronutrient intake and dietary changes during disease trajectory particularly with regard to nutrition impact symptoms (NIS) remains to clearly define in head and neck cancer (HNC) patients. The purpose of this study was to assess the micronutrient intake and dietary changes of head and neck cancer patients at three time points of their disease trajectory. Methods: HNC patients (n=63) completed a three- day dietary record and Head and Neck Symptom Checklist (HNSC) at three time points (baseline, post-treatment, follow up). Food categories were classified according to macronutrient content and culinary role, as well their percent contribution to overall caloric intake. Compliance with Canadian micronutrient DRIs was compared between patients stratified as non-ONS or ONS according to the percent of calories consumed from ONS. Results: Dietary patterns changed significantly from baseline to post-treatment and follow-up, as proportion of calories from milk, ONS and soup increased significantly whereas the percent of calories from grain, meat, dessert and oil and sugar decreased. The majority of patients failed to meet the Canadian RDA for vitamins C, D, E, folate, calcium, magnesium and zinc at each time point. Patients in ONS group had significantly higher intake of micronutrients at all three time points but lower protein intake at post-treatment compared to non-ONS group. ONS group had higher weight loss and NIS at post-treatment and follow up compared to non-ONS group. Conclusions: The results of this study showed HNC patients dietary changes over disease trajectory affects their nutrient intake. Inadequate micronutrient intake is common among HNC patients.

Poster Presentations

81. Phage-Encoded Libraries of Chemically Modified Peptide and its Application for Ligand Discovery

S. Ng and R. Derda
Chemistry

Phage display is the dominant technology for the discovery of biological drugs. Antibodies and peptide derivatives derived from such technology are the fastest growing contributors to FDA-approved drugs. Through the linking of each displayed peptide to its encoding DNA, phage libraries are several orders of magnitude large than chemical libraries, but still could be screened for a target in a very efficient way. However, the library is usually limited to 20 natural amino acids. Advances in bio-conjugation make it possible to introduce small molecule to the library, therefore amplifying the potential of phage display. We have generated hybrid carbohydrate-peptide library and demonstrated its application for the selection of potent inhbitors. We further expand our expertise to generate library of cyclic peptides displayed on phage. In this poster, I will describe the results of the selection aided by high-throughput DNA sequencing. I will also present my latest results in making cyclic peptide library and its derivatives. We believe that chemically modified phage libraries offer an untapped opportunity to rapidly enrich potent agonist or antagonist for challenging targets, such as carbohydrate-binding proteins and targets involving protein-protein interaction.

82. Dynamics of radiation-induced bystander signals

V. Olobatuyi
Mathematical and Statistical Sciences

It has been observed, in several in vivo and in vitro experiments, respectively, that un-irradiated cells located in the neighborhood of a region irradiated at low radiation doses exhibit some biological effects such as cell death, cell damage, e.t.c. which is similar to the direct effects of radiation on radiated cells. These effects are called radiation-induced bystander effects. Radiation-induced bystander effects are results of responses to signals emitted by the irradiated neighbors. The signals react with the DNA of nearby cells and consequently, these bystander effects are triggered. Radiation-induced bystander effects have implications for radiation therapy and radiation protection, and unfortunately, the dynamics of the causal signals are still largely unknown. In particular, we are interested in the dynamics of the signal's lifespan and the identification of the cell specific parameters that play key role in the lifespan's dynamics. We also seek to further understand the dynamics of signals' emission and spread in both one-time and fractionated radiation exposure. In this talk, we use continuum differential equation models to investigate these dynamics.

Poster Presentations

83. How does Methylene Quinuclidinone rescue p53 mutants?

S. Omar and J. Tuszynski
Oncology

The transcription factor, p53, plays an extremely major role in the regulation of the cellular machinery. Hence, the inactivation of p53 is an efficient strategy adopted by cancer cells to promote their survival and progression. In fact, p53 is the most mutated protein in cancer cells. Of the most frequent mutations in p53, are the single missense mutations at its DNA binding domain: R273H and R175H. A few molecules have been identified as rescuers that can restore wild type (wt) activity to mutant p53. APR-246 is the only rescuer that is currently in clinical trials. Lambert et al. have shown that APR-246 is a prodrug that is metabolized to give methylene quinuclidinone (MQ), which binds covalently to mutant p53. Also, Wassman et al. have shown evidence that MQ binds to p53 at Cys124. However, the exact effect of this covalent bond at Cys124 on the p53-DNA complex is still unknown. To understand this structural effect, we ran 750 ns molecular dynamics simulations of wt-p53, R273H-p53 (contact mutation), MQ-R273H-p53, R175H-p53 (structural mutation) and MQ-R175H-p53 each bound to DNA. Our models show disorientation between p53 and DNA in the mutant p53-DNA complexes compared to the wild type and drugged mutants. MM-GBSA calculations show that the two mutants have a marked increase in their enthalpic binding energy (EBE) compared to wt-p53. Although, the covalent binding of MQ to R175H-p53 greatly decreases its EBE to DNA, it does not have a significant effect on the EBE of R273H-p53. This suggests that MQ has a structural effect on p53-DNA complex. However, this effect cannot make up for the lost electrostatic interaction between the DNA and the mutated positively charged R273 in R273H-p53. Therefore, its EBE remains high. These results are critical for designing new and effective p53 rescuers.

84. Bcl-2 interacting killer (Bik) A prognostic indicator of breast cancer patient outcomes

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J. Hugh, J. Mackey, T. Mcmullen, and I. Goping
Biochemistry

Breast cancer is one of the leading causes of cancer-associated deaths in North American women. Cancer is a heterogeneous disease with many factors clouding accurate prediction of prognosis, treatment modalities and quality of life. Estrogen, progesterone and epidermal growth factor receptor expressions are routinely in clinical use as prognostic factors. Despite their success in guiding therapies, treatment failures leading to recurrent disease remain a clinical challenge, warranting discovery of novel prognostic factors with higher specificity and selectivity. Apoptosis and autophagy are cell death and cell survival pathways respectively that greatly determine neoplastic aggressiveness. BH3-only proteins are central regulators of both these pathways and hence offer promise of biomarker discovery. We investigated expression levels of BH3-only members in breast cancer patients and in particular Bcl-2 interacting killer (Bik) for its utility as a prognostic factor in breast cancer outcomes (disease free and overall survival). We analyzed mRNA levels of 5 BH3-only candidates alongside well-known clinicopathological variables in 176 breast cancer patients. Through univariate Cox analysis we found Bik and Bid mRNA expression and mitotic grade to be significantly associated with recurrence free survival. Interestingly Bik was the only independent variable retained upon multivariate Cox analysis. Further investigation against 3 anti-apoptotic Bcl-2 family members ruled out the possibility of stoichiometric compensation. Strikingly, uni- and multivariate analyses of Bik, Bcl-2 and Bad protein expression on patient derived tumor tissues also established predictive power of Bik being independent of Bcl-2 and a fellow BH-3 member Bad. This analysis indicates a clear association of Bik with poor patient prognosis. Additional investigation of Bik gene expression against autophagy biomarker ATG5 revealed a Bik: ATG5 correlation. Indeed a subset of breast cancer cell lines stably expressing Bik suggested high autophagy levels. Thus Bik may stimulate autophagy mediated survival pathways that contribute to clinical cancer aggressiveness.

Poster Presentations

85. Enhanced detection of cancer biomarkers in blood-borne extracellular vesicles using nanodroplets and focused ultrasound

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Electrical and Computer Engineering, Oncology

The feasibility of personalized medicine approaches will be greatly improved by the development of non-invasive methods to interrogate tumor biology. Extracellular vesicles shed by solid tumors into the bloodstream have been under recent investigation as a source of tumor-derived biomarkers such as proteins and nucleic acids. We report here an approach using sub-micrometer ultrasound contrast agent perfluorobutane nanodroplets and focused ultrasound to enhance the release of extracellular vesicles from solid tumors into the blood. HT1080-GFP fibrosarcoma tumors in chicken embryos were injected intravenously with nanodroplets and tumors were exposed to 30 seconds of ultrasound. The released extracellular vesicles in the blood were enumerated and characterized using micro-flow cytometry using green fluorescence to determine which vesicles came from GFP-expressing tumors. Only in the presence of nanodroplets could ultrasound release tumor-derived vesicles into the blood. A variety of biological molecules were successfully detected in tumor-derived extracellular vesicles, including fluorescent GFP protein, mRNAs and miRNAs. Sonication of HT1080 tumors released extracellular vesicles which contained detectable RAC1 mRNA with the highly tumorigenic N92I mutation known to exist in HT1080 cells. Applying ultrasound to HT1080 tumors also increased tumor-derived DNA in the serum by two orders of magnitude. The circulating tumor-derived DNA released into the blood by ultrasound aligned relatively evenly throughout the entire human genome as determined by deep sequencing. This work is the first demonstration of enhanced extracellular vesicle release by ultrasound stimulation and suggests that nanodroplets/ultrasound offers promise for relatively non-invasive genetic profiling of tumor phenotype and aggressiveness by stimulating the release of extracellular vesicles into the blood.

86. Non-viral (polymeric) delivery of combinational siRNAs against cell cycle and phosphatase proteins to prevent metastasis in breast cancer

M. Parmar, K.C. Remant, R. Maranchuk, H. Aliabadi, J. Hugh, and H. Uludag
Pharmacy and Pharmaceutical Sciences

Conventional breast cancer therapies have significant limitations that warrant development of new therapies. The siRNA-mediated silencing of a unique or over-expressed cell cycle proteins could lead to better control of tumor growth. Moreover, several evidences confirmed the role of protein-tyrosine phosphatases in metastasis. We hypothesize that dual siRNA delivery against a cell cycle protein (to decrease tumor cell growth) and a phosphatase protein (to decrease cell migration) may have a drastic impact to treat metastatic breast cancer. We initially confirmed the feasibility of delivering siRNA against CDC20, a key protein in cell cycle regulation using a non-metastasizing MDA-MB-435 cells in vitro and in vivo. Here, we performed siRNA delivery studies using metastasizing breast cancer cell-line MDA-MB-231. To deliver CDC20 siRNA effectively to MDA-MB-231, we synthesized a library of lipid-substituted polyethylenimines (PEI), and PEI substituted with linoleic acid (PEI-LA) was found to be the most effective delivery agent based on inhibition of MDA-MB-231 cell growth. To increase the stability of siRNA/PEI-LA complexes, hyaluronic acid (HA) was used as an additive or coating on complexes. HA additive was less toxic and inhibited cell growth significantly higher compared to complexes without HA. To identify siRNAs that were effective against cell migration, we screened siRNA library against 267 phosphatases for inhibition of cell growth and migration. Based on the library screening, siRNAs against PPP1R7, PTPN1, PTPN22, LHPP, PPP1R12A and DUPD1 decreased the migration of MDA-MB-231 cells significantly. These identified targets were then validated in vitro using individually prepared siRNAs. The combinational siRNA therapy has successfully decreased the growth as well as migration of MDA-MB-231 in vitro. This study confirmed the importance of CDC20 and several novel phosphatase targets to reduce metastasis of breast cancer. The non-viral delivery system described here could serve as a viable platform for delivery of multiple siRNAs against critical targets.

Poster Presentations

87. Theranostic evaluations of bioreductively-activated Tirapazamine (TPZ) prodrugs for the management of hypoxic solid tumors

S. Raman, P. Kumar, L. Postovit, and M. Weinfeld

Oncology

Hypoxic solid tumors are resistant to conventional radio- and chemotherapy (due to impaired drug delivery) interventions. Therefore tumor hypoxia presents significant human health challenges and contributes to poor overall survival of cancer patients. Tirapazamine (TPZ) is a highly potent hypoxia-selective clinical drug that was used in several clinical trials, but was withdrawn from the clinic due to its severe neurotoxic manifestations and poor patient population selection. We intend to pursue the translational explorations of glucose-TPZ-based conjugates and the corresponding radiohalogenated pharmaceuticals (that are being developed) to evaluate their potential in future clinical management of hypoxic solid tumors. Glucose moiety will facilitate their transport (through upregulated glucose transporters) in hypoxic cancer cells whereas TPZ will bioreductively activate in hypoxic atmosphere selectively and bind to cytoplasmic macromolecules therein to impart four-fold theranostic features. Healthy cells are oxygenated therefore the drug will not be retained therein, and minimal toxicity will be experienced by them. Overall, the goal is to carry out preclinical translational studies to validate the theranostic (therapy+diagnostic) potential of our conjugates. Initial studies are being carried out on selected cancer cell lines to test the hypothesis, followed by pre-clinical studies in hypoxic tumor-bearing animal models. Validation of the expression of GLUT-1 in hypoxic cancer cells has been done. Additional experiments to evaluate its chemo/ radiotherapeutic potential of hypoxic tumors are underway. Basically, validation of a series of theranostic studies will determine their anti-cancer potential and provide a basis for future clinical trials with curative response.

88. A phosphatidylserine-binding peptide to image tumour cell death in vivo

A. Perreault, M. Wuest, S. Richter, C. Foerster, C. Bergman, and F. Wuest

Oncology

Objectives: The ability to image tumour cell death in vivo using positron emission tomography (PET) would provide important information on cancer treatment efficacy. Phosphatidylserine (PS), an inner membrane phospholipid that is externalized during apoptosis, is a promising target for such an agent. Annexin V is often used to identify apoptotic cells in vitro; however, its use as an in vivo imaging agent is limited by its dependency on extracellular calcium and poor uptake into solid tumours. 14-mer PS binding peptide 6 (PSBP-6, sequence FNFRLKAGAKIRFG) could be an alternative for in vivo imaging of cell death, as it has better tissue penetration properties and does not require Ca2+ for binding to PS [1]. Methods: A radiometric PS-binding assay was used to determine PS-binding peptide potencies. PSBP-6 was conjugated with metal chelator 1,4,7-triazacyclononanetriacetic acid (NOTA) for radiolabelling with 64Cu. Plasma samples from mice injected with 64Cu-NOTA-PSBP-6 were analyzed by high performance liquid chromatography to determine in vivo stability of the radiopeptide. A cell assay was used to evaluate 64Cu-NOTA-PSBP-6 binding to EL4 lymphoma cells treated with camptothecin. Preclinical PET imaging studies were carried out on EL4 tumour-bearing mice treated with a chemotherapeutic mixture to examine the ability of 64Cu-NOTA-PSBP-6 to image chemotherapy-induced tumour cell death in vivo. Results: PSBP-6-based peptides displayed potencies in the micromolar range (IC50 6-600 M). 64Cu-NOTA-PSBP-6 was obtained in quantitative radiochemical yields, and displayed good stability in vivo. EL4 cells treated with camptothecin showed significantly higher (1.5-fold) binding of 64Cu-NOTA-PSBP-6 compared to untreated cells. When mice were treated with the chemotherapeutic mixture, a significant (1.3-fold) increase in EL4 tumour uptake of 64Cu-NOTA-PSBP-6 was observed at 5 min post-injection. Conclusions: 64Cu-NOTA-PSBP-6 shows promise as a Ca2+-independent PS-targeting agent for PET imaging of therapy-induced tumour cell death. References: [1] Xiong C, et al (2011) J Med Chem, 54, 1825-35.

Poster Presentations

89. Lipid Substitution on Low Molecular Weight Polyethylenimine for Combinatorial siRNA Delivery in Breast Cancer

S. Plianwong, R. Bahadur KC, C. Kucharski, T. Rojanarata, and H. Uludag
Chemical and Materials Engineering

Cationic polymer is a safe method for delivering siRNA since it has the ability to condense siRNA to nanosize and neutralize anionic siRNA without genetically affecting host cells. In this study, low molecular weight (MW 0.6, 1.2 and 2.0 kDa) polyethylenimine (PEI) was substituted by lipids (linoleic acid LA, alpha linoleic acid aLA, and cholesterol, Chol) via N-acetylation with amide or thioester linkages. The resultant polymers was evaluated in MCF7 cells with three different siRNAs targeting myeloid cell leukemia-1 (Mcl1), survivin and Signal Transducer and Activator of Transcription 5A (STAT5A). Based on inhibition of cell growth, four polymers from the polymer library (2.0 PEI LA 9, 1.2 PEI aLA 4, 1.2 PEI tLA 10 and 1.2 PEI taLA 6) emerged as effective carriers. They were subsequently used for combinational siRNA delivery. The combination of Mcl1/Survivin, Mcl1/STAT5A and Survivin/STAT5A decreased cell viability significantly. However, the combinations was mostly not significantly different from the single siRNA treatments (except 2.0 PEI-LA9/Mcl1/Survivin combination). The reduction of mRNA level of Mcl1, Survivin and STAT 5A was observed after 48 hr of transfection quantified by RT-qPCR. The combination of Mcl1/Survivin showed higher mRNA reduction than single siRNA. Interestingly, Mcl1 mRNA was increased in cells treated with Survivin or STAT5A siRNA, but not Mcl1 siRNA. In conclusion, lipid substituted low molecular weight PEI is able to deliver multiple siRNAs simultaneously, resulting in cell death and mRNA reduction. The combinatorial siRNA results suggest that Mcl 1/Survivin targeting is better approach to control the growth of MCF7 cells.

90. Photoacoustic and Ultrasonic Molecular Imaging of Cancer

R. Zemp, W. Shi, R. Chee, R. Paproski, and P. Hajireza
Electrical and Computer Engineering

I will present an overview of recent efforts to image cancer gene expression and profile tumor biomarkers using emerging photoacoustic imaging technologies. I will also highlight recent efforts on nanoscale multiplexable contrast agents for cancer molecular imaging and biomarker profiling.

Poster Presentations

91. Treating Bladder Cancer with Oncolytic Vaccinia Virus From Basic Discovery to Clinical Development

K. Potts, C. Irwin, N. Favis, R. Moore, D. Evans, and M. Hitt
Oncology

Introduction: Bladder Cancers (BCa) has a recurrence rate of up to 80% and many patients require multiple treatment strategies that often fail leading to disease progression. In particular, the current standard of care for high-grade disease, Bacillus CalmetteGurin (BCG), fails in around 30% of patients. Oncolytic viruses preferentially replicate in and lyse cancer cells while sparing normal cells. Virus replication requires an abundant supply of dNTPs, but because cellular enzymes that synthesize dNTPs are degraded at the end of Sphase, vaccinia virus (VACV) expresses its own biosynthetic enzymes including both I4L (large, R1) and F4L (small, R2) subunits of the heterodimeric ribonucleotide reductase. We have shown that deleting the F4L gene inhibits virus replication in normal cells while retaining replication proficiency in cancer cells. Results and Methods: We developed preclinical and clinical grade VACVs tagged with the gene encoding mCherry and deletions in F4L, J2R (viral thymidine kinase) or both. Cytotoxicity assays show a high degree of cell killing in infections with VACVs. Highly efficient VACV replication was seen in our BCa cells while limited replication was seen in normal bladder cells or primary fibroblasts. We show that our VACVs selectively replicated in both the orthotopic AY-27 immunocompetent and RT112-luc immunocompromised models causing significant tumor regression or complete ablation with no toxicity. Additionally, immunocompetent rats treated with the VACVs developed a protective anti-tumor immunity that was evident by tumor rejection upon challenge and by in vitro assays. Conclusion: Our data indicate that the VACVs have retained much of their replication proficiency and cytotoxicity despite deletions of critical viral replication genes. If successfully the VACVs could provide an essential line of therapy for patients that do not respond to BCG. This presentation will discuss the current status of our program and our progress towards a clinical trial in bladder cancer.t

92. Synthetic lethality of polynucleotide kinase/phosphatase with mitotic regulators

X. Qian, C. Lewis, and G. Chan
Oncology

Synthetic lethality result of simultaneous knockdown of two non-allelic, non-essential genes leading to cell death. The Weinfeld lab conducted a screen with 6961 siRNAs, searching for synthetic lethal partners of the DNA repair protein polynucleotide kinase/phosphatase (PNKP). Out of the 425 hits, there were a selection of mitotic regulators including WEE1, CDK1, MPS1, CENPE, TPX2, PKMYT1, PLK1, and AURKB that were potentially synthetic lethal with PNKP. We aim to verify the synthetic lethal relationship between these mitotic regulators and PNKP. We treated A549 lung carcinoma cells with inhibitors of mitotic proteins and observed their viability at various concentrations of the inhibitor. When treated with WEE1 and CDK1 inhibitor, no significant reduction of viability was observed in the CRISPR knockout PNKP A549 cells in comparison with the wild type control for either of the mitotic proteins. These observations provide evidence that PNKP is not synthetic lethal with WEE1 or CDK1 when using CRISPR knockdown A549 cells and chemical inhibitors. The validation of the synthetic lethality of the other mitotic proteins with PNKP is to be determined.

Poster Presentations

93. In vivo shRNA screen reveals KIF3B as a direct driver of metastasis

S. Raha, K. Stoletov, R. Paproski, D. Bond, D. Brown, and J. Lewis
Oncology

Metastasis is the leading cause of cancer-related deaths, yet there are no therapies that directly target this process. To identify potential therapeutic targets of metastatic dissemination, we recently completed the first whole human genome shRNA screen using a novel intravital avian embryo imaging platform developed by our group. More than 20 genes not previously linked to metastasis were identified, including KIF3B, which we determined is required for both in vivo cell migration and metastasis of human cancer cells in avian embryo and mouse models. KIF3B, a member of the kinesin family, is a (+)-end directed microtubule motor that targets the delivery of molecules from the cell interior to the cell periphery. Using an intravital imaging approach, we observed that cancer cells depleted of KIF3B form long cytoplasmic extensions but are unable to productively migrate. To this end, we investigated the mechanisms through which KIF3B promotes cancer cell motility. We found that cells depleted of KIF3B adhere differently to extracellular matrix via integrin-mediated focal adhesions. Additionally, in a pilot study we found that cancer cells depleted of KIF3B extravasate less than control cells in avian embryos. Overall, our study reveals a novel role for KIF3B in cell migration and metastasis which may ultimately lead to the development of specific anti-metastatic therapies.

94. Molecular insights into the mechanism of potential hypoxic radiosensitizers IAZA and FAZA

F. Rashed, A. Stoica, C. Ricardo, D. Macdonald, R. Siva, R. Fahlman,
L. Postovit, P. Kumar, and M. Weinfeld
Oncology

The hypoxic environment, a characteristic of many solid tumors, is known to select and promote growth of a subpopulation of tumor cells with increased proliferative and metastatic potential. Higher degrees of hypoxia are strongly correlated with lower survival rates in cancer patients. In our study, two hypoxic radiotracers, iodoazomycin arabinoside (IAZA) and fluoroazomycin arabinoside (FAZA), are currently being investigated for their potential (radio)-therapeutic role. Both compounds were able to restore the radiosensitivity of hypoxic cells to similar levels as normoxic cells. Understanding their mechanism of action at the molecular level should facilitate treatment by selecting chemo and/or radio-therapeutic regimens with synergistic effects. Here we show that the expression of HIF1-α transcription factor, a known promoter of hypoxic tumour growth and metastasis, is down-regulated by IAZA and FAZA. In addition, these compounds restored the level of radiation-induced double strand breaks under hypoxia to a similar level found in cells irradiated under normoxic conditions as demonstrated by the phosphorylated histone γ-H2AX. It therefore appears that these compounds are acting on transcription regulation through HIF1-a and enhancing DNA damage. Finally, using a click-chemistry approach, an azido conjugate of the drugs enabled us to isolate and identify their potential binding partners through mass spectrometry.

Poster Presentations

95. Stabilized radiolabelled bombesin peptides for targeting of gastrin-releasing peptide receptors in cancer

S. Richter, M. Wuest, C. Bergman, S. Krieger, B. Rogers, and F. Wuest
Oncology

Peptide receptor-based targeted molecular imaging and therapy of cancer is on the forefront of nuclear medicine preclinical research and clinical practice due to frequent overexpression of peptide receptors on tumor cells. The frequent overexpression of gastrin-releasing peptide (GRP) receptor in cancer prompted the development of radiolabeled bombesin derivatives as high affinity peptidic ligands for selective targeting of the GRP receptor. This study reports on the synthesis and radiopharmacological evaluation of metabolically stabilized bombesin analogues labelled with radionuclides fluorine-18 and gallium-68 for application in positron emission tomography (PET). Bombesin peptides displayed high inhibitory potency towards the GRP receptor (IC50 = 4.6 -16.5 nM) in PC3 prostate cancer cells. The 18F-labelling of bombesin analogue BBN2 via the two prosthetic groups [18F]SFB and [18F]FDG and the 68Ga-NOTA-Ava-BBN2 analogue were obtained in greater than 50% decay corrected radiochemical yield (RCY) with radiochemical purities of greater than 95%. 18F- and 68Ga-labelled BBN2 peptides showed high metabolic stability in vivo with greater than 45% of the radiolabelled peptide remaining intact after 60 min p.i. in mouse plasma. PET studies in PC3 xenografts demonstrated maximum tumour uptake after 5-10 min for [18F]FBz-Ava-BBN2 (SUV5min = 0.33), [18F]FDG-BBN2 (SUV5min = 0.56) and 68Ga-NOTA-Ava-BBN2 (SUV10min = 0.80). Elimination pathways of the bombesin analogue BBN2 can be rerouted towards a favourable renal clearance by using these different 18F-labeled prosthetic groups or a 68Ga-chelate modification. In conclusion, the 18F-carbohydrated bombesin [18F]FDG-BBN2 and the 68Ga-NOTA-Ava-BBN2 are superior PET radiotracer for molecular imaging of GRP receptor-positive prostate cancer and attractive candidates for translation into the clinical practice due to their greater tumor uptake level and favourable renal elimination pattern alongside with benefical metabolic stability in vivo. 68Ga-NOTA-Ava-BBN2 holds the potential of being developed into a theranostic agent for both diagnosis and treatment of cancer.

96. Targeting lysyl oxidase for molecular imaging in breast cancer

M. Wuest, M. Kuchar, S. Sharma, S. Richter, I. Hamann,
L. Vos, J. Mackey, F. Wuest, and R. Lser
Oncology

Lysyl oxidase (LOX) and its family members are copper-dependent extracellular matrix enzymes. Compared to normal breast tissue, LOX expression is elevated in breast cancer (BC), where it plays a central role in metastasis. The goal of the present translational research project was to visualize LOX expression in vivo in preclinical models of BC for molecular imaging. Tissue microarray (TMA) analysis revealed that mRNA of all LOX enzymes, except of LOXL4, were upregulated in human BC biopsy samples obtained from 176 BC patients. All three preclinical BC models (EMT6, MCF7 and MDA-MB231) were found to express LOX on protein level. Confocal microscopy and flow cytometry analysis with EMT-6 cells using a LOX-specific antibody and fluorescent-labeled peptide FITC-GGGDPKGGGGG-NH2 showed baseline expression of LOX under normoxia, which increased under hypoxic conditions. Positron emission tomography (PET) using radiolabeled peptide [18F]FBz-GGGDPKGGGGG-NH2 showed initial high tumor uptake after 5 min following continuous washout over 60 min post injection. Specific interaction of the radiopeptide with LOX in vivo was analyzed with the irreversible LOX inhibitor beta-aminopropionitrile (BAPN) in EMT-6 tumors resulting in a 30% reduction of tumor uptake. The present study highlights LOX as a novel and promising molecular target for PET imaging of BC. Moreover, TMA data from BC patient samples further underline LOX as an innovative drug target for BC patient management with metastatic disease.

Poster Presentations

97. Feasibility and Acceptability of Integrated Cardiac Rehabilitation in Outpatients Referred for Autologous Bone Marrow Transplantation

D. Rothe, N. Cox-Kennett, G. Gyenes, I. Paterson, I. Sandhu, C. Venner, and E. Pituskin
Nursing

Background: High-Dose Chemotherapy (HDCT) and bone marrow/hematopoietic cell transplantation (BMT) is established therapy for many malignancies. While advances in transplant practise have led to improved cancer-specific outcomes, HDCT negatively impacts healthy organ function via direct effects (i.e., high-dose cytotoxic injury to organ systems) and indirect effects(i.e., functional disability). The resulting cardiometabolic sequelae such as dyslipidemia, hypertension, diabetes, and weight gain (with lean body mass loss) contribute to the significantly increased rates of cardiovascular (CV) mortality and heart failure (HF) observed in HDCT survivors. Cardiac rehabilitation/secondary prevention (CR/SP) programs are a level one recommendation in multiple CV diseases, significantly reducing secondary CV risk and events. Currently the feasibility of integrating standard CR/SP programs in outpatients (PTS) referred to HDCT is unknown. Aim: To prospectively evaluate feasibility and acceptibility of routing referral to a CR/SP program in unselected lyphoma PTS referred for autologous HDCT/BMT Methods: Lymphoma PTS referred for HDCT/BMT were serially screened and referred to the CR/SP program. Baseline exercise testng was perfrmed prior to HDCT/BMT. Upon recovery (6 weeks) testing was repeated, and PTS were invited to articipate in a 6-week standard CR/SP program of exercise rehabilitation and CV risk reduction education Results: 20 PTS were referred for HDCT/BMT from January 1, 2015 to July 2015. All were referred to the CR/SP program. 100% underwent exercise testing, and all proceeded to BMT without adverse CV outcomes or mortality. High levels of satisfaction of CR/SP program components were reported. Conclusion: In unselected PTS, seamless integration of CR/SP within standard HDCT/BMT care is feasible and acceptable. We expect short term measurable impacts including reduced symptom burden and improved quality of life. Longer term impacts will evaluate CV morbidity and mortality. This work will inform patient-centered care and improve and survivorship care across the cancer continuum.

98. Exploring Novel Therapeutics in Pre-clinical Models of IBD

A. Said, Y. Fiteih, M. Kalla, M. Gordon, M. Salla, S. Fong, and N. Volodko
Biochemistry

Introduction: Inflammatory bowel disease (IBD) is a chronic intestinal disease characterized by inflammation of the gastrointestinal tract. RASSF1A or 1A is a tumour suppressor gene epigenetically silenced in human cancers and ulcerative colitis (UC) resulting in its functional inactivation. The genetic loss of 1A in our mice resulted in clinical symptoms of colitis and poor recovery following dextran sulphate sodium (DSS)-induced inflammation injury. Increased autophagic response was detected in the absence of Rassf1a. Furthermore, Rassf1a forms robust association with Nod2 upon muramyl dipeptide stimulation in colon cancer cells and 1A-/-Nod2-/- mice have higher survival rate and decreased disease severity upon DSS treatment when compared to 1A-/- mice. This would suggest that an active NOD2 pathway may be the driver of inflammation injury in DSS-treated Rassf1a mice. Nod2 is a pathogen recognition receptor known to be involved in both NF-kB activation and autophagic signalling. Upon DSS treatment, the lack of Rassf1a results in uncontrolled autophagic response via Nod2 that may be detrimental to epithelial repair and results in poor recovery from inflammation insults. RIPK2 is a member of receptor-interacting protein (RIP) family of serine/threonine protein kinases and a downstream adapter of the NOD2 signaling pathway. Upon the activation of RIPK2, NF-κB is activated and autophagy is induced.Method: 10-14 weeks old (early adolescence) Rassf1a and IL10 knockout mice were used. IBD was modelled by the addition of 3% w/v DSS into the drinking water for 7 days followed by fresh water for recovery.Results: Rassf1a can interfere with the ability of NOD2 to associate with the kinase, RIPK2. NOD2/RIPK2 signaling drives inflammation induced damage in DSS-treated Rassf1a-/- mice and its inhibition will promote increased survival. RIPK2 is up-regulated in many colorectal cancer patients and we can detect enhanced activity of RIPK2 in DSS-treated colon lysates and in human UC patient biopsies.

Poster Presentations

99. FasL is expressed preferentially over GzmB by CD8+ T cells responding to EG.7 lymphoma.

A. Scott and H. Ostergaard
Medical Microbiology and Immunology

CD8+ Cytotoxic T lymphocytes (CTL) kill cancer cells by several effector mechanisms, including degranulation of granzymes and perforin, as well as expression of Fas Ligand (FasL) on the CTL surface. These are independent in trafficking and CTL stimulus requirements; their relative importance in tumor control varies by tumor cell type and location. However, it is unclear if tumor-responding CTL indeed express FasL protein, and if this changes with tumor growth. As FasL is preferentially used by CTL under low antigen conditions in vitro, we hypothesize that FasL is expressed by CTL responding to tumors. We examined FasL expression by CD8+ T cells responding to the EG.7 lymphoma in vivo. Mice were injected with EG.7 cells either subcutaneously (SC) or intraperitoneally (IP). Mice receiving IP tumors received adoptive transfer of nave OT-I cells 24h before tumor injection. Peritoneal lymphocytes were collected from these mice at 7, 14, and 21 days post-injection. SC tumors were dissected over a range of volumes (100-900 mm3). All CTL were stained to detect cell-surface FasL or stored FasL and Granzyme B (GzB) by flow cytometry. Few cells in any activated CTL population expressed only GzB. In IP tumors, both antigen-specific and pan CTL populations increased coexpression of stored FasL and GzB over time. In SC tumors, the proportion of pan CD8+ T cells coexpressing FasL and GzB did not vary with tumor volume. Remarkably, the most frequent effector phenotype was stored FasL+GzB-. This was seen in antigen-specific cells of IP tumors as well as activated pan CTL populations of both IP and SC tumors. Tumor-infiltrating CTL in both models also expressed FasL on the cell surface at low levels. This suggests that FasL is actively used by CTL in the tumor microenvironment. Whether FasL is employed against tumor cells or the stroma remains to be determined. Future experiments will test the ability of therapeutically transferred FasL high/low CTL to clear established EG.7 tumors.

100. Gamma Delta T Cells and Nodal in the Breast Tumour Microenvironment

G. Siegers, G. Zhang, I. Dutta, H. Leong, A. Chambers, and L. Postovit
Experimental Oncology

Gamma delta T cells (GDTc) kill transformed cells, and increased circulating GDTc levels correlate with improved outcome in cancer patients. However, among a panel of tumor infiltrating lymphocytes (TIL), GDTc were deemed the most significant independent factor predicting negative clinical outcome in breast cancer. We hypothesize that GDTc become functionally altered by Nodal, an embryonic morphogen secreted by breast tumour cells in the hypoxic tumour microenvironment and implicated in aggressive disease. We have identified GDTc TIL and Nodal in breast cancer tissue sections. Under hypoxia compared to normoxia, GDTc viability and cell density increase, as does expression of the activating receptor CD56, the GDTc antigen receptor, HLA-I and CD95; conversely, the inhibitory receptor CD94 is down-regulated. Thus GDTc survive and proliferate in a hypoxic environment, and are armed with increased activating receptors implicated in cytotoxicity. Surprisingly, GDTc themselves express Nodal induced by hypoxia. Furthermore, short-term stimulation of GDTc with recombinant human Nodal results in tyrosine phosphorylation of a 50 kDa protein that we aim to identify. While primary human GDTc kill breast cancer cell lines, Nodal-expressing cells (231shC) resist GDTc killing compared to those in which Nodal has been silenced (231shN). Furthermore, Nodal overexpression confers greater resistance to GDTc cytotoxicity. Thus, Nodal appears to suppress the ability of GDTc to kill breast cancer targets. Tumour escape from GDTc killing may be further supported by downregulation of tumour antigen expression on target cells in hypoxic conditions, as we have observed for MICA/B on MDA-MB-231 cells. Chick chorioallantoic membrane assays reveal greater infiltration of GDTc into 231shN compared to 231shC tumours. We are now employing additional cell lines, and studying GDTc therapy and TIL in xenograft mouse models. Understanding the dynamic interplay between Nodal and GDTc infiltration in breast cancer lesions is of utmost importance to develop safe and effective GDTc immunotherapies.

Poster Presentations

101. Combining genome-wide association studies (GWAS) to predict the likelihood of radiation toxicity of a prostate cancer patient after treatment.

S. Singhal, N. Usmani, D. Broadhurst, A. Vega, C. West, S. Kerns,
B. Rosenstein, and M. Parliament
Oncology

Ionizing radiation therapy (RT) is a highly effective treatment modality for prostate cancer (PCa) and plays an integral role in the management of this disease. Although well tolerated by the majority of patients, up to 40% of PCa patients exhibit some form of normal tissue toxicity following RT. Therefore, we hypothesize that the use of predictive risk models incorporating genome wide association studies data from custom base arrays, patients clinical characteristics, and dosimetric variables will provide a robust tool to identify patients with PCa who are at high risk for significant late radiation-induced proctitis. The first step of this analysis is to merge the different genotyped data generated using the different platforms to increase power to detect associations.

102. Insight into Proteome Regulation by Micro RNAs

R. Piragasam, S. Chaulk, B. Millan, and R. Fahlman
Biochemistry

MicroRNAs (miRNA) are genome encoded small double stranded RNA which are the key regulators of gene expression and involved in various aspects of human development and diseases such as cancer. Many miRNAs are not individually expressed but are expressed as pri-miRNA clusters which can encode multiple miRNAs. An example is the human miR 23 24 cluster which encodes three miRNAs: miR-23a, miR-27a and miR-24-2. These three miRNAs have been individually implicated in diverse cellular processes such as regulating the cell cycle, proliferation, apoptosis, and are over expressed in many breast cancer cell lines but to date very little is understood about this miRNA cluster as a whole. In this study I report on a quantitative shotgun proteomic analysis to the global proteome changes in response to expressing the entire miR 23 24 cluster in HEK 293T cells, a cell line which does not normally express this miRNA cluster. The protein expression levels were quantified using label free approach called Extracted Ion Chromatogram (XIC). I will describe the technique and summarize our current data in changes of protein expression and cell functions affected by these microRNAs.

Poster Presentations

103. Polymeric Nano-Micelles for Delivery of a STAT3 Inhibitor to Melanoma

A. Soleimani, S. M. Garg, M.R. Vakili, R. Lai, and A. Lavasanifar
Chemical and Materials Engineering

Purpose: Signal transducer and activator of transcription 3 (STAT3) is constitutively activated in several human cancers. Despite acceptance of STAT3 inhibition as a promising strategy in cancer treatment, it has not been successfully translated to clinic, mostly due to toxicity and inefficient delivery of STAT3 inhibitors to tumors. S3I-1757 is an effective inhibitor of STAT3 dimerization that has shown activity against multiple cancer cell lines, but low water solubility and poor tumor selectivity have hampered its further development. The aim of this research was to develop polymeric micellar nano-formulations for the solubilization and selective delivery of S3I-1757 to melanoma tumors. Methods: Diblock copolymers of poly(ethylene oxide)-block-poly(caprolactone) (PEO114-b-PCL22) or PEO-b-poly(benzyl carboxylate-caprolactone) (PEO114-b-PBCL20) were synthesized and self-assembled to micelles. S3I-1757 was encapsulated in the micelles using co-solvent evaporation. Drug-loaded micelles were characterized for size, encapsulation efficiency and drug release. Free and micelle-encapsulated S3I-1757 was characterized for cytotoxicity against murine B16-F10 melanoma, and bone marrow-derived dendritic cells (BMDC)s by MTT assay. Inhibition of STAT3 transcriptional activity was assessed through measurement of VEGF in the cell supernatant by ELISA. The effect of free and encapsulated S3I-1757 in reversing the immunosuppressive effect of tumor supernatant on the stimulation of BMDCs was also investigated measuring over-expression of CD40, 86 and 80 on BMDC surface as well as IL-6 and IL-12 secretion by flow cytometry and ELISA, respectively. Results: PEO-PCL and PEO-PBCL micelles reached encapsulation efficiency of over 79% and average diameter of less than 55 nm after S3I-1757 loading. Free and encapsulated S3I-1757 showed similar IC50s against B16-F10, and similar dose dependent inhibition of VEGF production. Micellar S3I-1757 had significantly lowered cytoxicity against BMDCs compared to free drug and resulted in greater BMDC IL-12 production. Conclusion: PEO-b-PCL and PEO-b-PBCL micellar formulations of S3I-1757 are promising systems for the development of tumor targeted anti-STAT3 therapeutics in melanoma.

104. The Effect of Hormone Therapy on Quality of Life and Breast Cancer Risk After Risk Reducing Salpingo-oophorectomy: A Systematic Review and Meta-analysis

T. Siyam, S. Ross, S. Campbell, D. Eurich, and N. Yuksel
Pharmacy and Pharmaceutical Sciences

Objective: To identify, evaluate and synthesize evidence on the effect of hormone therapy (HT) on quality of life (QOL) and breast cancer risk, after risk reducing salpingo-oophorectomy (RRSO), in women who are carriers of BRCA mutations. Methods: We searched electronic databases including MEDLINE, EMBASE, CINHAL, and others, from inception to March 21, 2014, to identify relevant studies. Studies comparing the effect of HT to placebo, non-exposed group or baseline, in women who are carriers of BRCA mutations or who have high risk of breast or ovarian cancer and who have undergone a RRSO, qualified for inclusion. Primary outcomes included QOL and breast cancer. DerSimonian-Laird random effects method was used to calculate pooled weighted mean differences (WMDs). Generic inverse variance method was used to calculate pooled odds ratio (OR). Results: Of the 829 records identified, eight met our inclusion criteria. All studies were observational. Four studies assessed the effect on QOL. HT use was associated with improved QOL (WMD = 3.26, 95% CI = 0.96-5.56, P = 0.005). The risk of breast cancer was evaluated in 3 studies. Mean duration of follow-up was 2.6 years (range 0.1-19.1). The pooled OR showed a non-significant reduction in breast cancer risk with HT use (OR = 0.63, 95% CI = 0.28-1.40, P = 0.26). Conclusions: While cumulative evidence from our review suggests that the short term use of HT does not negate the breast cancer-risk reducing benefit of RRSO, there are too few long term studies to draw any strong conclusions. The need for future well designed randomized controlled trials for more established evidence is imperative.

Poster Presentations

105. Can HIF-1a silencing overcome hypoxia induced conversion of triple negative breast cancer to more tumorigenic and drug resistance phenotype?

H. Soleymani, N. Gupta, K. Gopal, R. Lai, and A. Lavasanifar
Pharmaceutical Sciences

Purpose: The long-term objective of this study is to assess the role of hypoxia inducible factor alpha 1 (HIF-1α) as a therapeutic target in triple negative breast cancer. For this purpose, we have first investigated the effect of hypoxia on the conversion of MDA-MB-231 cells to more tumorigenic and resistant phenotype. The effect of HIF-1α knock-down by small interfering RNA (siRNA) on this phenomenon was then investigated. Methods: Parental MDA-MB-231 and its two subsets sorted based on responsiveness to a Sox2 regulatory region (SRR2) reporter were used. The cell subset responsive to SRR2 reporter (RR cells) is found to be significantly more tumorigenic than the reporter unresponsive (RU) cells. MDA-MB-231 cells and its two subsets (RR and RU cells) were maintained under hypoxia (1-0.1% oxygen) at 37 C. Lipofectamine complexed HIF-1α siRNA was incubated with cells and the expression of HIF-1α and its downstream proteins were analyzed by immunoblotting. Conversion of RU to RR cells under hypoxia was investigated by measuring the level of GFP expression using Flow Cytometric analysis and luciferase assay. Lastly, the effect of HIF-1α knock down on the conversion of RU to RR cells under hypoxic condition was assessed. Results: Higher HIF-1α, p-Stat3, BAK and survivin expression were measured under hypoxia compared to normoxia in parental MDA-MB-231 cells and its two subsets (RR and RU cells). Successful knockdown of HIF-1α under hypoxia did not produce any significant effect on the expression of its down-stream proteins, e.g., BAK, MCL1, survivin, cleaved caspase 3, cleaved PARP, but gave rise to the expression of p-Stat3 and c-Myc. RU cells converted to RR cells under hypoxia. Unexpectedly, knockdown of HIF-1α under hypoxia increased RU to RR conversion. Conclusion: Hypoxic condition led to the over-expression of HIF-1α, generation of more tumorigenic and resistant phenotype in MDA-MB-231 cells. Unexpectedly, HIF-1α knockdown under hypoxia resulted in increased conversion of RU cells to more tumorigenic RR phenotype. This may be attributed to the compensating effect of other transcription factors overexpressed following HIF1-α knockdown.

106. Establishing an Experimental Model for Mesenchymal Stem Cell (MSC) Targeting of Renal Cell Carcinoma (RCC) in the Avian Chorioallantoic Membrane (CAM)

Z. Xu, H. Chen, D. Pink, K. Carmine-Simmen, J. Lewis, and R. Moore
Surgery

Metastatic RCC is a lethal urologic disease with 10 year survival less than 5 percent. Recent developments in cell signalling and small molecule therapy for mRCC have impacted progression free survival (PFS) only. High-dose IL-2 immunotherapy can produce durable complete responses (CR) but the response rate is low (16%), highlighting the need for novel therapy. Recently, MSCs were reported to traffic to murine RCC lung metastases via inflammatory signals, opening the potential of delivering tumour site-specific treatment. We hypothesize that human MSCs could target human RCC engrafted in the CAM of the avian embryo. In this project, we investigated potential use of the avian CAM model to study MSC RCC homing. This immunodeficient model is ideal for imaging tumour growth, and cell trafficking. We first transduced the 786-O RCC line to express green fluorescent protein (GFP) using lentiviral-based vectors. For the ex ovo model, fluorescent 786-O cells were initially injected into the CAM. Viable, vascularized 786-0 RCC tumours were observed in the CAM, 7 days post injection. Concurrently, human adipose derived MSCs were isolated from patient perirenal fat, cultured, and characterized using flow cytometry. The primary culture was observed to be CD73, CD90, CD105, CD44, CD166 positive and CD45, CD19, CD106, CD146 negative, consistent with MSC expression. MSCs, labelled with a fluorescent plasma membrane stain, were injected IV into RCC tumour-bearing chicken embryos. Injected MSC movement was monitored using intravital imaging via confocal microscopy. Injected commercial and patient-derived human MSCs were observed in proximity to the RCC tumour consistently over periods of 18 hrs and 3 hrs respectively. However, MSCs were not observed to specifically traffic to the RCC tumour. Our results demonstrated that both viable RCC tumours and MSCs were established and imaged in the CAM model, setting the stage for future studies exploring MSC tumor homing.

Poster Presentations

107. Intravital discovery of miRNA drivers of metastatic cascade in human cancers

L. Willetts, K. Stoletov, E. Woolner, and J. Lewis
Oncology

Metastasis is the leading cause of cancer patients death. MicroRNAs (miRNAs) have been implicated as key regulators and biomarkers of cancer metastasis, yet the identification of miRNA/mRNA networks that directly drive metastasis in vivo has been cumbersome. Our lab recently conducted the first in vivo whole human miRNAome screen for miRNAs that drives invasive cell migration, which is critical to metastatic cascade. This screen uncovered a panel of individual miRNAs that are critical in regulating invasive cell migration. Interestingly, we noted some significant bias in the molecular pathways that these miRNAs target. Specifically, we found that the majority of metastasis-regulating miRNAs are predicted to target TGFb signaling, phosphatidylinositol signaling, histone methylation and actin cytoskeleton remodeling pathways. mRNA target analysis of these screen-identified miRNAs (DIANA, TargetScan) showed an enrichment in therapeutically targetable genes: cell surface receptor kinases (such as BMPR2), phosphoinositide kinases (such as PI3K and PIKFYVE) and histone methyl-transferases (e.g., WHC1L1, SETD2 and KMT2C). Mechanistically, we found that these miRNAs control the ability of cancer cells to efficiently invade along the vasculature. Cancer cells with deregulated pro-metastatic miRNA expression failed to efficiently attach to the vasculature and form persistent and directional cell membrane protrusions. Next, we propose to expand these findings to comprehensively map the network of screen-identified miRNAs and their therapeutically relevant mRNA targets that drive key metastatic steps of primary tumor invasion and metastatic lesion formation. We will use crosslinking and sequencing of miRNA/mRNA hybrids to determine direct targets of miRNAs that drive the formation of tumor metastatic lesion. Subsequently, we will use a range of unique in vitro and in vivo approaches developed by our group to validate the role of these identified miRNA/mRNA networks in human cancer invasion and metastasis, with focus on those mRNAs for which clinically relevant inhibitors/therapeutics are currently available. The discovery of pro-metastatic miRNA/mRNA networks will greatly aid in the development of novel, personalized, multi-parametric and cancer metastasis-specific biomarkers and therapeutics.

108. Evolving Pathology in PTLD: A Case Series

R. Stubbins, A. Peters, R. Lai, and J. Preiksaitis
Medicine

Post-transplant lymphoproliferative disorders (PTLDs) are classified as early PTLDs (E-PTLD), polymorphic PTLDs (P-PTLD), monomorphic PTLDs (M-PTLD) and classical Hodgkins Lymphoma PTLD (HL). These entities are felt to represent a disease continuum, with a precursor-product relationship, though they are morphologically and clinically distinct. However, this process remains poorly understood, and limited evidence exists to support this hypothesis. We report a series of nine cases extracted from a PTLD database with recurrent disease episodes that evince an apparent evolution in their morphologic categorization between episodes. All patients identified were high risk for PTLD, with multiple identifiable predisposing factors. Presentations varied from isolated lymphadenopathy, to gastrointestinal involvement to pulmonary involvement. Four of the patients were deceased at the time of acquisition, though only one directly from PTLD. Eight of the nine identified patients developed E or P-PTLD lesions that preceded the subsequent M-PTLD at similar tissue sites. Two of the cases had metachronous P and M-PTLD lesions. The M-PTLD subtypes were variable, including DLBCL, Burkitt, T-cell, extramedullary plasmacytoma and three cases of classical Hodgkins lymphoma. These cases suggest that E and P-PTLD may act as common precursor lesions to the development of the M-PTLD variants, and that the PTLD classification schema represents different stages of a common underlying pathology.

Poster Presentations

109. Immune Responses Associated with Disruption of Different DNA Repair Pathways in Colorectal Cancer

S. Sumi, C. Mowat, A. McNamara, and K. Baker
Oncology

Colorectal cancer (CRC) is the third most common cancer worldwide. CRC is a very heterogeneous disease associated with genomic instability that is the key molecular feature of tumorigenesis. Two different types of genomic instability have been characterized namely Microsatellite Instability (MSI) and Chromosomal Instability (CIN). In our lab we are working on disruption of different DNA repair genes involved in both MSI and CIN colorectal cancer. The mucosal immune system exists as a protective shield against gut microbiota and has also developed numerous regulatory mechanisms that lead to tolerance to large amounts of foreign material. It is believed that the intestine is more susceptible to developing cancer than other organs because the mucosal immune system is even less likely to react to altered tumor proteins than immune cells of other organs. The main focus of our study is to investigate how CRC escape immune detection in the tumor microenvironment. We are also determining what kind of immune responses are associated with disruption of different DNA repair pathways that produce different patterns of genomic instability. We are using the genome engineering tool prokaryotic type II CRISPR-CAS9 system to introduce genetic alterations of different DNA repair genes e.g. MLH1, MGMT, MUTYH, APC, RAD51, MSH2, MSH3, K-RAS, POLE, and P53.We will then investigate how this changes the expression of immune regulatory genes in three-dimensional cultures of intestinal epithelial cells as organoids. We believe that our work on cell culture, animal models and patient samples will give us valuable information for targeting a therapeutic intervention because it will increase the ability of immune system to detect cancer cells as foreign. Besides chemotherapy and radiation therapy, our research will contribute to discovering new adjuvant therapeutic strategies for the treatment of CRC.

110. Structural Insight Into BLM Recognition by TOPBP1 in Maintenance of Genome Stability

L. Sun, Y. Huang, W. Niedzwiedz, and J.N. Glover
Biochemistry

The Bloom's syndrome protein (BLM) is a member of the conserved RecQ helicase family. Mutations in BLM can lead to Bloom's syndrome, a disease characterized by growth retardation and predisposition to cancer. For years, researchers hypothesis that Topoisomerase binding protein 1 (TopBP1), an important mediator protein in DNA replication checkpoint control, can potentially interact with BLM through its 5th BRCA1 C terminal (BRCT) domain. However, the mechanism of this interaction remains unclear. Recently, the Chen lab suggested that BLM recognition by TopBP1 mainly involves BLM pSer338. However, the Niedzwiedz lab suggested pSer304 of BLM is more essential for its interaction with TopBP1 both in vitro and in vivo. We are interested in identifying the primary target site of TopBP1 BRCT5 on BLM and understanding how this interaction is regulated in cell. We used fluorescence polarization (FP) to show that TopBP1 BRCT5 binds with high affinity to peptides corresponding to the pSer304 region of BLM but not the pSer338. To better visualize this interaction, we determined the crystal structure of mammalian TopBP1 BRCT4/5 in complex with a pSer304 containing BLM peptide, FVPPpS304PE using X-ray crystallography. The peptide is docked against TopBP1 BRCT5 with its pSer304 binds the conserved phosphate binding pocket of BRCT5, while the residues N-terminal to the pSer dock against the β2- β3 loop of BRCT5. Intriguingly, the BLM peptide binds in an opposite orientation compared to the pSDpTD peptide derived from the DNA double strand break mediator MDC1. Using site directed mutagenesis and FP, we demonstrate the recognition of BLM peptide involves a complex set of hydrophobic and electrostatic interactions while a lower affinity, electrostatically-driven interaction is involved with MDC1. These structural insights provide a new platform for potential anticancer inhibitory drug design targeting TopBP1 interactions with BLM.

Poster Presentations

111. The oncogenic FOXM1 transcription factor in cancer treatment: is it a selective 'druggable' target?

S. Dakhili, R. Aguayo-Ortiz, and C. Velzquez
Pharmacy

Genome-wide gene expression profiling of human cancers has consistently identified the Forkhead box M1 (FOXM1) transcription factor as one of the most commonly activated genes in cancer cells. Also, abnormal activation of the FOXM1 gene is regarded as one of the hallmarks of chemoresistant cancer cells including (but not limited to) liver, breast, gastric, skin, renal, pancreatic, brain, prostate, ovarian, and colorectal. Accumulating evidence suggests that targeted FOXM1 inhibition may be a promising strategy to treat many types of cancer, suggesting that FOXM1 inhibitors may be clinically useful drugs. In this regard, there are no reported drugs that directly bind to and inhibit the FOXM1/DNA binding domain in cancer cells. As part of an interdisciplinary research project aimed to validate the FOXM1 transcription factor as a drug target, we recently carried out a series of molecular modeling protocols to determine possible binding sites in the FOXO/DNA and FOXM1/DNA domains. We have identified a series of drug molecules that could potentially, and selectively, inhibit the oncogenic FOXM1 while sparing the tumor suppressor FOXO proteins. We will discuss the Medicinal Chemistry concepts used to generate essential information about the structural requirements that are essential for a drug to inhibit the transcriptional activity of FOXM1. We hope to stimulate discussion about the druggability of this protein, and the implications of new FOXM1 inhibitors in clinical practice.

112. Doxycycline increases the plasma membrane expression of lipid phosphate phosphatases and decreases plasma LPA concentrations: implications for cancer therapy

X. Tang, Y. Zhao, J. Dewald, J. Curtis, and D. Brindley
Biochemistry

Tetracyclines have been used for decades as antibiotics. Independently of their bactericidal activity, tetracyclines also inhibit the activities of matrix metalloproteinases (MMPs), they promote apoptosis and they have anti-inflammatory effects. These properties give tetracyclines the potential to be used as antineoplastic agents. Our previous work demonstrated that increasing the low activity of lipid phosphate phosphatase-1 (LPP1) in cancer cells decreased tumor progression and metastasis by degrading lysophosphatidate (LPA) and attenuating its ability to signal through its receptors. This study describes a novel way to increase LPP activity. We found that 1mg/ml doxycycline, tetracycline orminocycline significantly increased the expressions of the lipid phosphate phosphatases (LPPs) on the plasma membranes of several transformed and non-transformed cells. This increased the dephosphorylation of extracellular LPA in cell culture. Also, treating rats with 50mg doxycycline/kg/d accelerated the clearance of exogenous LPA from circulation. Doxycycline increased the expressions of LPP1-3 through decreasing their rates of degradation and this did not depend on inhibition of MMP activity. The doxycycline-induced increasein LPP activity suppressed LPA-stimulated growth of mouse 4T1 cell breast cancer cells in 3-D culture. It also attenuated LPA-induced migration of human MDA-MB-231 cells. In a syngeneic mouse breast cancer model, 50mg doxycycline/kg/d significantly decreased plasma LPA concentrations by about 30% and reduced breast tumor size by about 40%. The present work demonstrates for the first time that tetracyclines increase ecto-LPP activities. This property could provide a new strategy for attenuating LPAsignaling in cancers and other inflammatory conditions.

Poster Presentations

113. Small Hydrophobe Substitution on Polyethyleneimine for Effective Plasmid DNA Delivery into Breast cancer cells

B. Thapa, S. Plianwong, R. Bahadur KC, and H. Uludag
Pharmacy and Pharmaceutical Sciences

Cancer gene therapy has been intensively developed using cationic polymers as non-viral vectors due to their safety and facile chemistry to tailor their properties. We modified 1.2 kDa PEI (1.2PEI) using small hydrophobe propionate (PrA, CH2-CH3) and explored for DNA delivery to breast cancer cells. Substitution efficacy of PrA onto 1.2PEI was increased with feed ratio (PrA/PEI) and DNA binding efficacy of PEI-PrA was decreased in proportion with PrA substitution as a consequences of -NH2 consumption and/or steric hindrances. Hydrodynamic size of polyplexes was identical irrespective to PrA substitution, but surface charge initially increased with PrA substitution and later decreased at high PrA substitutions. Cellular toxicity of the polymers was increased with PrA substitution but the polymers still displayed less toxicity compared to 25 kDa PEI (PEI25). pDNA uptake and GFP transfection efficiency in both MDA-231 and MCF-7 was significantly increased with optimal PrA substitution (0.5-1 PrA/PEI) while polymers with the highest substitution and parent polymer were ineffective. Importantly, transfection efficiency of PEI-PrA1 (PrA/PEI equal to 0.76) was higher than long lipid (C18) grafted 1.2 PEI and comparable to PEI25. In addition, PEI-PrA1 showed higher transfection than PEI 25 at day 7 and 14, showing a stable transfection. Substitution of small hydrophobe onto 1.2PEI enhanced transfection efficiency in breast cancer cells, but excess substitution (more than 1.2 PrA/PEI) was detrimental, emphasizing the importance of balancing polymer hydrophobicity for effective gene delivery.

114. Ligand-induced growth of CD36-Fyn clusters induces signaling

J. Githaka, A. Vega, M. Baird, M.. Davidson, K. Jaqaman, and N. Touret
Biochemistry

Nanoclustering is emerging as a key organizational principle of membrane proteins. Using superresolution imaging, we investigated the molecular organization of the clustering-responsive receptor CD36 and its downstream effector Fyn in response to thrombospondin-1 (TSP-1), an anti-angiogenic ligand that promotes endothelial cells apoptosis. At steady state, CD36 receptors pre-exist in nanoclusters (diameter of about 100 nm). Fyn is already present in these clusters, and its N-terminal membrane targeting domain is sufficient for this association. Even if already associated, we found that Fyn only becomes activated activated upon TSP-1 binding through an enhancement of CD36-Fyn clustering, forming larger and denser clusters. These enhancements are supported by the actin cytoskeleton and the presence of plasma membrane cholesterol as their perturbation abolishes signaling. Our data demonstrate cooperation between cholesterol-dependent domains and the cortical actin cytoskeleton in the reorganization of the receptor-effector pair CD36-Fyn that enables signaling during TSP-1 stimulation.

Poster Presentations

115. Characterization of constitutive heterochromatin breakdown during breast cancer progression

S. Ugochukwu, G. Shi, K. Missiaen, K. Ziegler, and A. Underhill
Oncology

Epigenetic marks are emerging as biomarkers for cancer diagnosis and treatment, including disease of the breast. This reflects the critical role chemical modification of DNA and histones has in the control of genome structure and function. In this context, trimethylation of lysine 9 on histone H3 (H3K9me3) and lysine 20 on histone H4 (H4K20me3) is associated with constitutive heterochromatin within pericentromeric regions and both modifications are required for chromosome integrity. Despite this requirement, only H4K20me3 displays widespread loss in cancer, suggesting it has a unique tumor suppressive function. Our research is focussed on understanding the mechanistic basis of this activity using breast cancer as a model system. To this end, we have characterized H4K20me3 in breast cancer progression models that include human (MCF-7 and MDA-MB-231) and murine (NMuMG and 4T1) cell lines, as well as in tumor samples and metastases from the PyMT mouse. In each case, we observed decreased H4K20me3 in association with more advanced disease, which was most pronounced in less differentiated cells of PyMT tumors. We also initiated bioinformatics analyses in the same model systems using a combination of proteomic and gene expression data. This revealed a global decrease of the pericentromeric heterochromatin proteome in more aggressive disease, together with marked changes in transcript levels for proteins that either directly or indirectly modulate H4K20me3 levels. Collectively, these studies provide a strong framework for understanding H4K20me3 loss in breast cancer and defining its role in pathogenesis.

116. Fibronectin Modified Surfaces for Chronic Myeloid Leukemia cell adhesion to Evaluate Influence on Sensitivity to Leukemia Therapies

J. Valencia-Serna, P. Chevallier, G. Laroche, and H. Uludag
Biomedical Engineering

Tyrosine kinase inhibitor therapies against Chronic Myeloid Leukemia (CML) inhibit the production of fast-dividing cells. However, a small portion of insensitive cells impedes the eradication of the disease. This aberrant process is partially attributed to an altered adhesion via integrin receptors of CML cells to fibronectin (FN) present in the bone marrow, which may confer protection to most molecular drug therapies. To understand the importance of this adhesion process, this study was designed to immobilize FN on functionalized PTFE films, and investigate the response of attached K562 cells to exploratory siRNA therapy. Adhesion assays revealed that FN grafting with sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate) (SMPB) allowed much higher cell density than Clean PTFE, and FN grafting with glutaric anhydride (GA). MTT analysis showed the initial cell numbers were similar for adsorbed and grafted FN; however, the cell growth on subsequent days was higher on films with grafted FN via SMPB or GA. SiRNA treatment consisted of particles of GFP-siRNA and PEI2-LA polymer (linoleic acid-modified polyethyleneimine). The GFP silencing on GFP-K562 cells attached on PTFE+SMPB+FN was evaluated and compared to that of cells adhered (if any) on Clean PTFE films, and cells in suspension. The GFP silencing of cells on Clean PTFE was -7.63.5%, whereas the silencing for cells attached on PTFE+SMPB+FN films was 25.118.9%, and for the cells in suspension was 36.49.0%. FN-films grafted with SMPB were the most efficient promoting cell adhesion and growth. Moreover, adhesion of leukemia cells through FN did not affect significantly the siRNA silencing efficiency in comparison with the cells grown in suspension, suggesting that the siRNA delivery treatment with lipid-polymeric carriers can transfect leukemia cells regardless of their suspension or adhesion state. The FN grafted through SMPB on PTFE films is potentially a good model for assessing the effects FN-mediated CML adhesion have on the response to siRNA and other therapies.

Poster Presentations

117. The oncogenic FOXM1 transcription factor in cancer treatment: is it a druggable target?

C. Velazquez, A. Elshaikh, A. Tabatabaei, K. Barakat, and J. Ussher
Pharmacy and Pharmaceutical Sciences

Genome-wide gene expression profiling of human cancers has consistently identified the Forkhead box M1 (FOXM1) transcription factor as one of the most commonly activated genes in cancer cells. Also, abnormal activation of the FOXM1 gene is regarded as one of the hallmarks of chemoresistant cancer cells including (but not limited to) liver, breast, gastric, skin, renal, pancreatic, brain, prostate, ovarian, and colorectal. Accumulating evidence suggests that targeted FOXM1 inhibition may be a promising strategy to treat many types of cancer, suggesting that FOXM1 inhibitors may be clinically useful drugs. In this regard, there are no reported drugs that directly bind to and inhibit the FOXM1/DNA binding domain in cancer cells. As part of an interdisciplinary research project aimed to validate the FOXM1 transcription factor as a drug target, we recently carried out a series of molecular modeling protocols in which we have determined the binding energies of 3,323 FDA-approved drugs within the FOXM1/DNA binding domain. As a result of this study, we have identified six promising virtual reference drugs with significant binding affinities. Furthermore, we are studying the selectivity of these lead molecules toward different (but structurally related) FOX proteins. We will present a Medicinal Chemistry protocol used to generate essential information about the structural requirements that are essential for a drug to inhibit the transcriptional activity of the oncogenic FOXM1, while sparing FOXO3 (an important tumor suppressor protein). We hope to stimulate discussion about the druggability of this protein, and the implications of new FOXM1 inhibitors in clinical practice.

118. Role of β-catenin/Active β-catenin in Osteosarcoma progression

G. Venkateswaran, L. Xia, and S. Persad
Pediatrics

Background: Osteosarcoma (OS) is the most common primary bone malignancy with high incidence in children and adolescents. Although the overall survival rate has increased in OS patients over years, it still remains as one of the childhood cancer with lowest overall survival rate. Wnt signaling pathway is one of the signaling pathways that is deregulated in most cancers. Although a number of studies have shown deregulation of this pathway to be implicated in OS, role of β-catenin (key regulatory component of Wnt signaling) in this cancer is not clear. While some studies support the involvement of β-catenin in OS, others show the contrary. All these studies investigate the role of β-catenin rather than Active Beta Catenin (ABC) which is a fraction of β-catenin that is transcriptionally active. ABC transcribes genes involved in cell proliferation, invasiveness and hence promotes cancer. Therefore in our study we are interested in investigating the role of β-cat/ABC in OS progression. Methods: We used two pairs of cell lines that represents OS progression: Saos2, Saos2-LM7 (aggressive cell line derived from SaOS2), HOS and HOS-143B (aggressive cell line derived from HOS). We used Ezrin, MMP2 and MMP9, some of the currently known markers that are known to be involved in OS progression as measure of aggressiveness in the cell lines used. Western blotting and Immuno-fluorescence techniques were used for determining the cellular levels and localization of β-cat and ABC. Results: Our results showed the aggressive OS cell lines (SaOS2-LM7 and HOS-143B) to express higher cellular levels of ABC compared to their less aggressive parent cell lines SaOS2 and HOS. However there was no significant change in the levels of β-cat with aggressiveness. Conclusion: Our results suggest a strong association between ABC and OS progression. We therefore propose that ABC might potentially have a role in progression of OS.

Poster Presentations

119. High expression of SFRP1 in melanoma is associated with poor patient prognosis and invasive cellular phenotypes

K. Vincent and L. Postovit
Oncology

Secreted frizzled-related protein 1 (SFRP1) is a putative tumour suppressor gene, which has been shown to be epigenetically silenced in a variety of cancers. However, the role of SFRP1 in melanoma tumourigenesis is not clear. We found that SFRP1 is expressed at significantly higher levels in metastases compared to primary tumours. Analysis of a clinical dataset of primary melanomas showed that high SFRP1 expression was significantly associated with poor disease-free and distant metastasis-free survival. SFRP1 expression was determined to be 4.4 fold higher in samples characterized by an invasive versus a proliferative state (as defined by phenotype-specific mapping). In melanoma cell lines, expression of SFRP1 was inversely correlated with promoter methylation. To examine the functional impact of this protein, we generated SFRP1 knockout cell lines using a TALEN-mediated gene editing approach. Knockout cell lines displayed reduced invasive capacity, consistent with the clinical observations. Our study implicates SFRP1 in invasive phenotypes and demonstrates, for the first time, that high SFRP1 expression can serve as an independent predictor of poor survival in melanoma.

120. From Basic to Translational Research: Understanding and Targeting Infiltrative Tumour Cells in Malignant Glioma

K. Vo, R. Burchett, M. Brun, and R. Godbout
Oncology

Background: Malignant glioma (MG) is the most deadly type of brain cancers because of the infiltrative nature of the tumour cells. Nuclear factor I (NFIA, NFIB, NFIC, and NFIX) have been implicated in the regulation of genes involved in MG cell migration. A putative NFI target genes, CAST, encodes calpastatin, an endogenous inhibitor of calpain proteases. Objective: CAST was selected for analysis because calpains regulate the activity of calcineurin, a phosphatase that dephosphorylates and activates NFI. The main objective of this project is to study the calpain-calpastatin-NFI axis and its effects on MG cell migration and survival. Methods: Physical interaction between NFI and CAST was confirmed in vitro by gel shift assays and in vivo by chromatin immunoprecipitation. We also knocked down different combinations of NFI and examine changes in RNA and protein levels of calpastatin, calpain 1, calpain 2, and calcineurin. MG cell migration and survival in response to Calpain Inhibitor I was evaluated by live cell imaging and clonogenic assay, respectively. Results: I have validated the binding of NFI to CAST. NFI depletion, specifically NFIC and X, results in upregulation of CAST RNA levels, but not calpain 1 and 2. NFI depletion does not alter calpastatin protein levels in MG cells with hyperphosphorylated (inactive) NFI, but increases calpastatin in MG cells with hypophosphorylated (active) NFI. Similarly, NFI depletion in the latter leads to the accumulation of inactive calpain 2 and uncleaved calcineurin. Regardless of NFI status, MG cells show a dose-dependent reduction in migration and survival in response to Calpain Inhibitor I. Conclusions: A positive feedback loop mediated by calpastatin may exist between NFI and the calpain pathway. This axis may increase both MG cell migration and survival. Therefore, calpain antagonists including ALLN could represent a novel class of chemotherapeutic agents for MG treatment.

Poster Presentations

121. Exploring MOAP-1 and RASSF1a methylation and expression in cancer

N. Volodko, M. Salla, and S. Baksh
Pediatrics

Introduction: Modulator of apoptosis 1 (MOAP-1) is a BH3-like protein that forms a complex with the umor suppressor RASSF1A (Ras association domain family protein 1A) to promote apoptosis through Bax activation. RASSF1A loss of expression due to promoter specific methylation has been reported in numerous cancers. However MOAP-1 mRNA expression changes (if any) in cancers is poorly studied. In this study, we have explored MOAP-1 and RASSF1A mRNA expression in cancer cell lines and primary tumors (breast, thyroid and Hodgkins lymphoma [HL]). Methods: DNA and RNA were extracted from cell lines and tumors using All prep DNA/RNA Mini Kit. Total RNA was converted into cDNA by using the High Capacity cDNA Reverse Transcriptase Kit. MOAP1 and RASSF1A mRNA expression was studied by qRT-PCR. DNA was bisulphite modified and methylation was studied by pyrosequencing. We developed pyrosequencing assays to interrogate methylation status of 32 CpGs in RASSF1A promoter and 19 CpG s in MOAP-1 promoter Results: We confirmed that RASSF1A promoter was heavily methylated in solid tumors but not in blood cancers (except in HL). RASSF1A methylation generally resulted in lower mRNA expression. Interestingly, despite that MOAP-1 promoter contains CpG island with high CpG density, it was found not methylated in cancer cell lines, but its expression linked to RASSF1A expression. Conclusions: Our data suggest that MOAP-1 expression level is not regulated by its promoter specific methylation, but it is linked to RASSF1A mRNA expression. Funding Sources: AI-HS and the Stollery Childrens Hospital/Hair Massacure Department of Pediatrics Donation Fund

122. Microarray Prediction of Response to Nivolumab, Ipilimumab, or Combined Therapy in Subjects with Previously Untreated, Locally Advanced or Metastatic Melanoma

L. Vos, M. Smylie, J. Walker, K. Famulski, and P. Halloran
Oncology

Until recently there have been few options for the treatment of melanoma in the advanced/metastatic setting. Advances in both immune and genetically targeted treatment approaches have revolutionized the spectrum of treatment options for melanoma patients over the last several years. Immune-based therapy of cancer through altering immune checkpoint inhibition involves inhibition of regulatory cell surface molecules which act normally to dampen or modulate T-cell activation. Melanoma lesions often contain a high number of infiltrative T-cells specific to melanocyte tumor associated antigens such as MART1, gp-100, and tyrosinase. There is a clinical impact of the location and type of immune cells within colorectal cancer and this may also occur in melanoma. Not all lymphocytes are created equal, and to discern one subset from another is technically challenging and requires a high degree of labor and skill.

Poster Presentations

123. The Effects of Nodal on Human Fibroblast Activation

Zhang, Piaseczny, Findlay, Jewer, Vincent, Liu, Postovit
Oncology

Metastasis is the main cause of cancer death for breast cancer patients. However, the causative factors and the mechanisms by which cancer cells spread and metastasize remain unclear. Some studies have shown that activation of cancer associated fibroblasts (CAFs) may be pivotal in providing the microenvironment favoring tumor progression and metastasis. In comparison to healthy fibroblasts, CAFs express alpha-Smooth Muscle Actin (a-SMA), some Desmin and Connective Tissue Growth Factor (CTGF) concomitant with increased invasion, migration, and proliferation. Studies from our research team and others have demonstrated that Nodal, a secreted embryonic morphogen, promotes breast cancer tumorigenesis, and is present in the stroma. We herein hypothesize that Nodal causes the activation of stromal fibroblasts toward an activated phenotype. We tested this hypothesis by exposing primary human foreskin fibroblast (HFF) to recombinant human Nodal for varying periods of time and at varying concentrations. Western blotting from HFF exposed to Nodal for 1hour, demonstrated that ERK1/2 and SMAD2/3 are both phosphorylated in response to Nodal. These results demonstrate that HFF can respond to Nodal. A-SMA protein levels were elevated in HFF treated with Nodal for 3 days, and this increase lasted between 48 to 72 hours after the Nodal containing medium was removed. Accordingly, RT-PCR showed a dramatic increase in the expression of major fibroblast activation markers, a-SMA and CTGF. Using Boyden chamber assays, we determined that Nodal promotes HFF migration and invasion dose dependently between 0ng and 100ng Nodal; and cell proliferation assay showed that Nodal causes a modest increase in HFF proliferation. Collectively, this data suggests that Nodal induces fibroblast activation. Future studies will examine the consequence of this activation on breast cancer metastasis.

124. Role of DEAD Box Protein 1 in Alternative Splicing

Y. Wang, L. Li, and R. Godbout
Experimental Oncology

DEAD box proteins are RNA helicases that are involved in all aspects of RNA metabolism. DEAD Box 1 (DDX1) is a RNA unwinding protein that was originally cloned from a subtracted retinoblastoma cDNA library and found to be amplified in a subset of childhood tumours. It has previously been shown that the Drosha-DGCR8 complex competes with the spliceosome complex in the processing of pre-mRNAs, thereby altering RNA splicing. DDX1 has been reported to function as a regulatory component of the Drosha-DGCR8 complex. We hypothesize that knockdown of DDX1 reduces Drosha-DGCR8 complex activity, thereby interfering with its ability to compete with the spliceosome complex, and leading to changes in the alternative splicing of a subset of pre-mRNAs. We have carried out gene expression microarray analysis of HeLa cells transfected with scrambled siRNA versus DDX1 siRNA. The most differentially expressed gene between these two populations of cells was apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3H (APOBEC3H). As the APOBEC3H gene has 4 splice variants (variants 1 - 4), we selected this gene for further analysis. Upon transfection of HeLa cells with siRNAs targeting both DDX1 and Drosha, we observed a reduction in APOBEC3H variant 4 and an increase in APOBEC3H variant 1 compared to cells transfected with scrambled, DDX1 or Drosha siRNA alone. These data suggest that DDX1 and Drosha may indeed play of role in APOBEC3H alternative splicing. We are currently pursuing these experiments by co-IP in order to further understand how DDX1 interacts with the Drosha-DGCR8 complex, thus shedding light on DDX1s role in alternative splicing.

Poster Presentations

125. Notch activation induces EMT in epithelial ovarian cancer cells

J. Zhou, A. Azad, S. Jain, H. Yu, Z. Xu, R. Godbout, and Y. Fu
Oncology

The Notch signaling pathway determines cell fate by regulating multiple cellular processes. Notch activation can be either oncogenic or tumor suppressing depending on the tissue context. Activation Notch plays an oncogenic role in epithelial ovarian cancer (EOC). However, the underlying molecular mechanisms are not fully understood. Epithelial-mesenchymal transition (EMT) is a critical event in the progression of EOC. This study was designed to investigate whether activation of Notch1 induces EMT and whether Notch1 and transforming growth factor beta (TGFbeta), a potent EMT inducer, interacts in the context of EMT in EOC cells. We stably transduced human EOC cell line OVCA429 and SKOV3 cells with the intracellular domain of Notch1 (the constitutively active form of Notch1) to activate Notch1. Our results showed that activation of Notch1 induced EMT in these cells as evidenced by downregulation of epithelial marker E-cadherin and upregulation of mesenchymal markers (Slug and Snail) as well as morphological change of cells to a more spindle-like shape. In accordance with the mesenchymal phenotypes, activation of Notch1 rendered EOC cells more resistant to carboplatin, the first line therapeutic agent to treat EOC. Interestingly, Notch1 activation increased TGFbeta signaling by upregulating the expression of TGFbeta and TGFbeta type I receptor and activation of Notch was partially required for TGFbeta-induced EMT in EOC cells. On the other hand, TGFbeta treatment increased the expression of Jagged1 (Notch ligand) and HES1 (Notch target gene) in EOC cells. Functionally, the combination of Notch1 activation and TGFbeta treatment was more potent in promoting migration of EOC cells than either stimulation alone. Our results demonstrate that Notch and TGFbeta forms a reciprocal positive regulatory loop and collaborate in regulating EMT in EOC cells, suggesting that Notch and TGFbeta signaling could be potential therapeutic targets to treat this deadly disease.

126. cMyc characterizes a small cell subpopulation in ALK positive anaplastic large cell lymphoma that carry higher tumorigenicity and chemotherapy resistance.

C. Wu, H. Zhang, N. Gupta, R. Lai
Lab Medicine and Pathology

We have previously identified two phenotypically distinct cell subpopulations in ALK+ anaplastic large cell lymphoma based on their differential response to a Sox2 reporter (SRR2), that cells responsive to the reporter (RR) are more tumorigenic and chemo-resistant than cells that are reporter unresponsive (RU). The determinant factor(s) between RR and RU cells are still elusive. Our bioinformatics analysis of the SRR2 sequence suggests that cMyc may be an important co-factor. In support of this concept, we found RR cells have a substantially higher expression of cMyc and phosphorylated cMycS62, as well as more Sox2/cMyc-SRR2 probe binding. Specifically, cMyc not only promotes Sox2 binding to the SRR2 probe, but also maintains the expression of Sox2 in NPM-ALK/STAT3 dependent manner. Correlating with these observations, cMyc inhibition significantly inhibited the clonogenicity of RR cells, and sensitized RR cells to doxorubicin. Furthermore, we found Wnt canonical pathway and MAPK pathway, which are known to upregulate cMyc expression/activation, were highly activated/expressed in RR cells, as compared to RU cells. Intriguingly, we observed a positive regulatory loop in RR cells, in which Sox2 up-regulates Wnt1 and Wnt2B, both of which can activate the Wnt canonical pathway and cMyc. The significance of cMyc in this context was further highlighted by that RU cells with cMyc over-expression capture the similar biochemical/biological characteristics with RR cells. Lastly, we found cMyc is only highly expressed in a small number cells of ALK+ALCL patient tumors (n=50), and the expression of cMyc is also highly correlated with active ?-catenin. In conclusion, cMyc is a key regulatory factor in the determination of RU/RR dichotomy, which is linked to tumorigenic potential and chemo-resistance in ALK+ALCL.

Poster Presentations

127. Investigating the roles of Sox2 and KLF4 in stem cell DNA damage response

M. Wu and A. Gamper
Oncology

The DNA damage response (DDR) in eukaryotes has evolved to manage constant exogenous and endogenous threats to genome integrity, and its unsurprising that downstream signaling in DDR is specific to plasticity and cycle state of the cell, and to the type of damage received. Pertaining to this, DDR in stem cells is believed to differ from that in differentiated somatic cells, leading to the hypothesis that there may be an interaction between DDR and stem cell renewal signaling. Elucidation of the potential crosstalk would provide important insights into ageing, have implications for stem cell applications and promote our understanding of carcinogenesis. Sex-determining region Y-box protein 2 (Sox2) and Krppel-like factor 4 (KLF4) are components of an ensemble of transcription factors able to induce pluripotency in differentiated cells. Previous work has shown that KLF4 is a high turnover protein thats stabilized by methylation following ionizing radiation (IR), whereas the regulation of Sox2 after DNA damage is uncharacterized. Our preliminary data indicate that Sox2 levels are significantly depleted in cancer cell lines 12 hours post-IR, and that depletion is mediated by proteasomal degradation. Furthermore, treatment with an ATM kinase inhibitor appeared to inhibit IR-induced depletion of Sox2, suggesting a link between ATM signaling and Sox2 regulation. In order to elucidate Sox2 and KLF4 regulation after DNA damage, we intend to explore their interacting partners by utilizing proximity-dependent biotin identification (BioID) and affinity purification. BioID allows for the identification of transient interaction partners in vivo using a promiscuous biotin ligase mutant, BirAR118G, fused to the bait. Affinity purification of epitope tagged proteins allows us to identify more tightly binding partners. We have established cell lines with tetracycline-inducible Sox2 or KLF4 fused to BirAR118G and a FLAG tag. Mass spectrometric analysis of interacting proteins will give insight into Sox2 and KLF4 regulation and function.

128. The role of JunB transcription factor in the pathobiology of ALK+ ALCL.

Z. Wu, J. Bacani, and R. Ingham
Medical Microbiology and Immunology

Activator Protein-1 (AP-1) family transcription factors regulate many normal cellular activities and their abnormal expression has been associated with various malignancies. My project focuses on the function of the AP-1 transcription factor, JunB, in the T cell lymphoma, Anaplastic Lymphoma Kinase-positive, Anaplastic Large Cell Lymphoma (ALK+ ALCL), where JunB is highly expressed. To investigate the function of JunB in the pathobiology of this lymphoma, we generated stable knock-downs of JunB with shRNAs and examined their effect on cell growth. We found that JunB is essential for the tumour cell proliferation because JunB knock-down resulted in a decreased proliferation rate; however, we observed no effect on apoptosis in the JunB knock-down cells. To further investigate JunB function in ALK+ ALCL, we performed microarray experiments comparing the JunB knockdown cells and control cells. Many genes identified in JunB knockdown cells are genes characterize ALK+ ALCL from normal T, suggesting JunB contribute to the transcriptional profile of ALK+ ALCL. Gene ontology analysis of our microarray data suggests that JunB may participate in immune evasion in this lymphoma, given that 20% of the genes with altered expression in the JunB knock-down cells were related to natural killer (NK) cell- mediated cytotoxicity. In support of this notion, preliminary results demonstrated that JunB knock-down cells are more vulnerable to NK cell-mediated killing than cells expressing control shRNA. In summary, we show that JunB promotes tumour cell proliferation, influences the expression of genes that characterize this lymphoma, and protects tumour cells from immune surveillance.

Poster Presentations

129. Medical-Legal Collaboration: Lawyers Role in Advance Care Planning

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Oncology

Background: The medical and legal fields coalesce around advance care planning (ACP). There is an opportunity for the legal community to be a stronger partner in the uptake of ACP beyond personal directive completion. Analysis is required of gaps in resources and supports to help lawyers promote and be engaged in ACP conversations. Aims: To assess barriers and facilitators to lawyers involvement in ACP and, in collaboration with the legal community and other ACP stakeholders, catalogue, review and develop ACP resources for lawyers to help them promote and be engaged in ACP conversations. Methods: Three workshops for researchers (6), lawyers (19), clinicians (3), and other stakeholders (8) from the legal and medical communities were held in Edmonton, Calgary and Red Deer to discuss lawyers roles, needs and barriers regarding ACP. These workshops brought together key stakeholder groups who have been working in parallel and have a keen interest in improving the uptake of ACP, but have otherwise had few opportunities to connect e.g. the Canadian Bar Association, Legal Aid, legal education organizations, academics, government representatives, and patient advisors. The outcomes of the meetings include: the development of a survey for distribution to all practicing Alberta lawyers, an outline of the contents of ACP legal resources, establishing roles and responsibilities around compiling the resources, and the development of a plan for dissemination. Results: A description of the meeting results, and the ACP legal resources compilation, development and dissemination will be presented. Discussion/Conclusion: The legal community is well placed to facilitate comprehensive ACP. ACP legal resources and inter-professional collaboration have the potential to enable lawyers to improve the uptake and quality of ACP.

130. The effect of Advance Care Planning documentation, discussions, and programs on healthcare costs: a systematic review

A. Chiu and K. Fassbender

Oncology

Advance Care Planning (ACP) encompasses discussion and documentation of patients future health care wishes. Previous reviews on the economic impacts of ACP have been limited in scope or have not provided quantitative synthesis of results. The aim is to assess the effects of ACP participation on healthcare resource use, as defined by monetary value, from all perspectives. An electronic database search was undertaken to identify empirical studies which include an intervention involving discussion, documentation, or participation in an ACP intervention program, and a comparator group. Search terms on costs, cost-effectiveness, and cost-benefit, were included with the intention of retrieving all studies with economic measures. A total of 236 articles were full-text screened for eligibility. The 36 studies meeting the inclusion criteria varied in study design (experimental or observational) and setting, whether or not only decedents were sampled, and ACP indicators used18 were documentation-based, 2 were discussion-based, 14 were for both documentation and discussion or institutional ACP programs, and 2 involved proxy measures. Studies varied in length of data collection, period for cost or charge assessment from 48 hours up to 36 months. Four studies accounted for or published costs of the ACP intervention. Twenty-eight studies showed decreased charges or costs in the ACP intervention groups, 6 showed cost increases, and 2 showed no effect or inconclusive results. Given significant heterogeneity among the types of resources identified in studies and the measures used to compute the published cost data, costs can only be compared across studies in a few cases. Nonetheless, the synthesis of the published quantitative data suggests that ACP engagement leads to reduced resource use. While there are potential resource savings with ACP in inpatient, outpatient, and other (home, long term, and hospice) care settings, there was no evidence on the impacts on out-of-pocket and private costs.

Poster Presentations

131. Identification and implementation of indicators to monitor successful uptake of Advance Care Planning in Alberta: a Delphi study (Phase I).

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Oncology

Introduction: In April 2014, Alberta Health Services and Covenant Health implemented Advance Care Planning (ACP) and Goals of Care Designation (GCD) across Alberta. This is a formal way to register patients opinion on care details, especially in emergency, when communication with the patient is impossible. Now there is a need to find methods of characterizing the quality of its functioning in a large, complex, multi-sector health care system. Objective: To identify the most informative indicators to monitor ACP/GCD across Alberta. Methods: 132 potential indicators were found in the literature through systematic review and environmental scan. A Delphi consensus-based approach is used to evaluate, reduce and refine them. We performed one face-to-face meeting and three Delphi rounds. The topics were: defining domains for the indicators (18 IOM?Donabedian), reduction and refinement of the list of indicators, determining most appropriate care settings. Results: Preliminary mapping of the indicators to the domains has selected 54 indicators with highest level of agreement, which were used in Delphi rounds. R.1. Invited 73 panellists, 16 completed responses. No consensus in 5 domains, therefore, 26 indicator(s) with highest evaluation remained. An additional 5 indicators suggested by panellists, 3 continued to subsequent round. R.2. Invited 72 panellists, with 9 completed responses evaluated. The resulting 18 distinct indicators used Round 3. R.3. Invitation for Round 3 was sent to 62 specialists from 7 types of care settings, with 24 complete responses. Results show indicators covered all levels of details and setting types; none of the indicators received too low applicability score. Conclusion: The results allow us to proceed to Phase II: defining data sources, testing and validating the ACP/GCD indicators.

132. Learning From a Learning Needs Assessment

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Background: There is growing recognition that palliative and end of life care occurs in many clinical settings, and nursing staff require basic competencies in end of life care regardless of their clinical setting. Moreover, there is a desire to design and provide palliative education appropriate for adult learners. Objectives: The primary purpose of the survey was to examine gaps in competencies that are presently outlined in several regulatory and educational documents. Secondly, we wished to examine learning needs and preferences. The analysis should help with future planning of an educational strategy for nursing staff across many clinical settings. Design: Competency-based, cross-sectional survey of nursing staff. CASN, CLPNA, Alberta HCA Curriculum was used for competency comparison. Participants: Registered nurses, licensed practical nurses, healthcare aides at healthcare organizations providing acute and continuing care services. Methods: Web and paper based surveys. Descriptive and correlative statistics used for analysis of 193 surveys. Results: Knowledge was influenced by continuing education and negatively correlated with end of life care instruction in initial nursing education programs. There was no difference between knowledge levels of the different nursing groups (RN, LPN, HCA) Conclusions: The relationship between formal instruction and knowledge is consistent with results from previous uses of the survey (continuing education has higher correlation with knowledge than initial education). It is unclear if established competencies accurately reflect competencies performed in practice. We need to establish expected levels of competency across clinical areas and consider evaluation of outcomes beyond individual learners.

Poster Presentations

133. Early palliative care and its translation into oncology practice in Canada:barriers and challenges

K. Fassbender and S. Watanabe
Oncology

This article reviews the progress Canada has made integrating palliative care into oncologic practice. Key clinical practice guidelines have influenced and have been translated into Canadian oncology policy and operations. Comprehensive accreditation standards exist to guide oncology practice in institutional and ambulatory care settings. Common barriers and challenges are discussed: education and attitudes, compassion fatigue, terminology, paucity of research, aggressive cancer care, and organization and operational considerations. As a result eight made-in-Canada innovations emerged and are described. Lessons learned and recommendations describe a plan for action.

134. The economic consequences associated with screening for distress and an integrated symptom relief service for cancer patients

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Oncology

Purpose. We prospectively compared the costs of outpatients before and after implementation of an integrated symptom relief service (ISRS) consisting of distress screening and outpatient management (psychosocial treatment, pain and fatigue clinics, nutrition services and palliative care consults) of patients presenting to a tertiary cancer setting with a head and neck or neurological cancer diagnosis. Patients and Methods. In this study we prospectively evaluated public and private consequences for both patients and caregivers using the Health Services Inventory (HSI), an encounter-based semi-structured interview tool. Clinician in-service sheets, patient information sheets, user manual, codebook and a medical organizer (a memory aid to reduce recall bias) were employed. Prior index month resource use was obtained through telephone interviews taking approximately 10 minutes. Results. A total of 247 patients (114 before, 133 after) reported 978 (588, 390) health care encounters in the prior month. Health care encounters declined significantly from before to after, as did patient encounter duration. Over 90 percent of patients relied on transportation and had at least one caregiver accompany them to all medical appointments. Additionally, patients experienced greater visit-related out-of-pocket costs associated with their visits during the after period. Statistical analysis shows that the data sets have a distribution of costs close to lognormal with a few outliers (5 and 7 respectively) most likely originating from another lognormal distribution corresponding to a different and considerably more costly type of treatment like long hospital admission. After removal of the outliers, comparison of the sample means shows statistical significance of difference in mean costs between the two samples ($p=0.03$). The reason is that after the psychological help the proportion of patients with low-cost types of treatment increased by about 10%. Conclusion. The ISRS program results in significant cost-shifting of Cancer Care from both public and private perspectives.

Poster Presentations

135. Advance Care Planning / Goals of Care Designation (ACP/GCD): Prevalence in Health Care Settings

A. Winkler, C. Brenneis, and K. Fassbender
Palliative Institute

In 2014, the Advance Care Planning/Goals of Care Designation (ACP/GCD) project was implemented across Alberta to assist clinicians when discussing and defining goals of care with their patients. The Advance Care Planning (ACP) Tracking Record documents ongoing reflection and communication between health care providers and patients and their families. The Goals of Care Designation (GCD), which is documented on a Physician Order Form, specifies the focus of care, medical interventions, transfers and locations of care. In 2015, Covenant Healths Palliative Institute conducted a chart audit to evaluate the implementation of the ACP/GCD policy at all 17 Covenant Health sites. 888 charts, which were distributed across health care sectors and all of Albertas health regions, were examined. Nearly two thirds of the charts audited had a GCD order, while two out of five had an ACP Tracking Record with at least one completed entry. The audit clearly indicates that uptake of both Tracking Record and GCD Order differ by sector. In acute care sites, half of the charts included GCD Order Forms, while in long-term care facilities nine out of 10 patients had the order present in their charts ($\chi^2(3)=158.648$, p=0.000). ACP Tracking records mirror this trend; they were present in 6% of the charts in acute care settings and in four out of five charts in long-term care facilities ($\chi^2(3)=277.523$, p=0.000). Our audit also shows that although ACP conversations are taking place, they are not always being documented in the ACP tracking records. In nearly 30% of the charts, progress notes indicate that these exchanges took place, but they did not appear in a Tracking Record. Our research indicates that barriers to uptake of ACP Tracking Records and GCD Order Forms in acute care settings have to be identified with the goal of increasing their use. In addition, continued education is required to encourage clinicians to document conversations in accordance with policy.

136. Pursing Excellence in End-of-Life Care: Review of a Clinical Pathway

K. Huebert, A. Winkler, C. Brenneis, and K. Fassbender
Palliative Institute

Deficiencies in end-of-life care have lead to the designing of clinical pathways. At Covenant Health, the Palliative Care Pathway was implemented in 2011 as a standard of care for those residents in long-term care facilities who are in the last hours to days of life. The Palliative Care Pathway helps health care providers to facilitate optimal symptom management. It addition, it ensures that information is shared with other health care providers, the residents, as well as their families. One of the principle aims of the pathway is to provide high quality end-of-life care and support to the residents and their family. We describe findings based on a 2015 quality assurance review of the Covenant Health Palliative Care Pathway. It examines the current state of the pathways implementation and use with reference to best palliative care practices. We reviewed 30 charts at 3 different sites. The analysis focuses on assessment and reassessment, communication with families and team members, planning care, documenting care provided, care after death, and management of symptoms. Implementation rates ranged from 18 57% of deaths. For the vast majority of patients (83%), the pathway was in place for the expected period of time of 1 to 4 days. For all patients, the initiation of the pathway was discussed with families, suggesting excellent communication at this stage of care. Medications ordered indicate good comfort care. For example, all patients with indication of pain or respiratory congestion had medications ordered for these indicators. Limitations of the study include that no consensus has been reached on what constitutes an appropriate benchmark for rate of implementation. In addition, variances, which are used to provide and document individualized care, have not been sufficiently conceptualized.

Poster Presentations

137. ACP CRIO: a bold, innovative knowledge translation research program studying a province-wide implementation of Advance Care Planning and Goals of Care Designation

N. Hagen, J. Simon, and K. Fassbender
Oncology

Advance Care Planning (ACP) is a process of reflection on and communication of a persons future healthcare preferences. ACP encourages dialogue between a patient, his/her family, and the healthcare team that can guide medical decision making even when a person becomes incapable of consenting to or refusing healthcare. ACP programs and policies are being implemented across healthcare systems around the world including in Alberta, Canada. Alberta Health Services (AHS) is the major publicly funded comprehensive health care organization for the four million residents of the province of Alberta. Goals of Care Designation is a made-in-Alberta medical order used by healthcare providers to describe and communicate the general aim or focus of care. In 2014, AHS implemented a multi-sector, provincial policy for ACP/GCD across all AHS facilities. ACP CRIO is a bold, innovative knowledge translation research program that has partnered with AHS to prospectively study the system-wide uptake of ACP/GCD, and its impact on the healthcare system. Five-year funding for ACP CRIO is from Alberta Innovates Health Solutions Collaborative Research and Innovation Opportunities (CRIO) competition. ACP CRIOs purpose is to determine how to optimally implement a formalized ACP framework across a large population and throughout a complex, multi-sector healthcare system. We have applied the knowledge-to-action cycle to support adoption and use of ACP/GCD across Alberta, through four research activities designed to identify: (1) Local barriers and facilitators to uptake of ACP/GCD; (2) Effective tools for education and engagement of stakeholder groups in ACP/GCD, adapted to the local environment; (3) Informative indicators to monitor uptake of ACP across the healthcare system, and how they can guide continuous improvement of the ACP implementation strategy; (4) Economic consequences of ACP implementation. Preliminary outcomes from this program of research will be presented.

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