



CRINA
Cancer Research
Institute of Northern Alberta

RESEARCH DAY 2016

“Biobanking and Translational Medicine”

Saturday, Nov 12th 2016
University of Alberta
CCIS 1-440



**UNIVERSITY OF
ALBERTA**

WELCOME



Dear CRINA Research Day Attendee:

Thank you for joining us at the third annual CRINA Research Day. Last year, at our second 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, with Cancer Biobanking as a featured theme. This year, we have continued to provide trainees with an opportunity to organize the program and present their work orally to our cancer research community at the University of Alberta. 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 University of Alberta cancer research strengths in terms of research excellence and available infrastructure and platforms, with plans to build on these strengths 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 the 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,

Dr. Lynne-Marie Postovit
Co-director, Basic Research,
Cancer Research Institute of
Northern Alberta

Dr. David Eisenstat
Co-director, Applied
Research, Cancer Research
Institute of Northern Alberta

MESSAGE FROM THE DEAN



Cancers have become the leading cause of premature death in North America, producing more deaths than heart disease, vascular disorders and stroke combined. Almost half of the North American population will develop cancer in their lifetimes. Furthermore, Canada's new cancer case rates are projected to increase by more than 50 per cent by 2030.

In Alberta, cancer occurrences reach an appalling number of more than 16,000 per year, and death rates are more than 6,000 per year. Lung cancer is the leading cause of cancer death in both Alberta men and women, and the most common cancers for males and females are prostate cancer, and breast cancer.

As an academic community it is our commitment to lead our significant efforts against cancer towards a healthy and productive population by improving cancer diagnosis, primary and secondary cancer prevention, and developing novel treatment approaches that can be rapidly introduced to patients through clinical trials in the academic and community setting.

In April 2014, the University of Alberta established the Cancer Research Institute of Northern Alberta (CRINA), gathering talented clinicians and cancer researchers from all affiliated teaching hospitals, departments and faculties. CRINA's research programs, aligned with adult cancer and malignant blood disorders clinical trials provided at the Cross Cancer Institute and the University of Alberta Hospital, as well as pediatric cancer clinical trials based at the Stollery Children's Hospital, are setting a new standard for cancer centres across Canada.

By leveraging the province's current research base towards greater competitiveness for major provincial and national awards and grants, we want to place Alberta among the global leaders in cancer research.

Richard Fedorak
Dean, Faculty of Medicine & Dentistry
University of Alberta

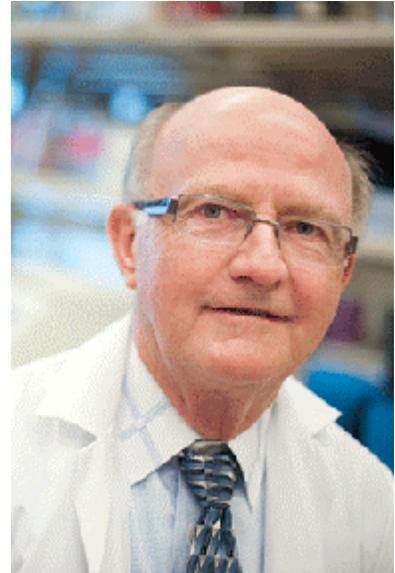
MESSAGE FROM THE VICE PRESIDENT (RESEARCH)

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 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 continues to demonstrate its 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 A. Babiuk
Vice-president (Research)
University of Alberta



PROGRAM

8:00 AM – 8:20 AM **BREAKFAST & REGISTRATION** CCIS PCL Lounge

8:20 AM – 8:30 AM **WELCOME ADDRESS** CCIS 1-440

Dr. David Evans, FoMD Vice-Dean Research
On behalf of Dean Fedorak

SESSION I: BIOBANKING AND NEURO-ONCOLOGY CCIS 1-440

Chair: Dr. Shairaz Baksh, Co-Chair: Zack Breckenridge

8:30 AM – 9:00 AM

Keynote Address, Dr. Jennifer Chan
Associate Professor, Departments of Pathology, Oncology, and
Clinical Neurosciences
Deputy Director, Charbonneau Cancer Institute
Kids Cancer Care Foundation Chair in Pediatric Oncology
Research
University of Calgary
*Oligodendroglioma: from pattern recognition to molecular
mechanisms*

9:00 AM – 9:15 AM

Dr. Ralf Schirmmacher, Associate Professor
Oncologic Imaging, Medical Isotope Cyclotron Facility
*Silicon-based 18F-radiochemistry for imaging tumors of
neuroendocrine origin with positron emission tomography*

9:15 AM – 9:30 AM Dr. David Eisenstat, Professor and Co-Director, CRINA
Muriel & Ada Hole Kids with Cancer Society Chair in Pediatric
Oncology
Departments of Pediatrics, Medical Genetics, and Oncology
*Characterization of Ganglioglioma as a Neurodevelopmental
Disorder: A Case of Arrested Development?*

9:30 AM – 9:45 AM Saket Jain, PhD Student
Department of Oncology
*Title: Role of Transcription factor AP-2 in Glioblastoma
Pathogenesis*

9:45 AM – 11:00 AM **POSTER SESSION 1/SNACKS** CCIS PCL Lounge
ODD NUMBERED POSTERS

SESSION II: CANCER BIOLOGY CCIS 1-440
Chair: Dr. Basil Hubbard, Co-Chair: Gareth Armanious

11:00 AM – 11:15 AM Dr. Todd McMullen, Associate Professor
Division of General Surgery, Department of Medicine
Division Director of Surgical Oncology, Cross Cancer Institute
*PDGFR α regulates follicular cell differentiation driving treatment
resistance and disease recurrence in papillary thyroid cancer*

11:15 AM – 11:30 AM Dr. Ismail Hassan Ismail, Assistant Professor
Department of Oncology
Cross Cancer Institute
Targeting DNA Repair Pathways for Myeloma Treatment

11:30 AM – 11:45 AM Dr. Kristi Baker, Assistant Professor
Department of Oncology
Control of anti-tumor immunity by DNA repair defects in colorectal cancer

11:45 AM – 12:00 PM Dr. Anahita Mojiri, Postdoctoral Fellow
Department of Medicine
Functional assessment of Von Willebrand factor (VWF) expression by cancer cells of non-endothelial origin

12:00 PM – 12:40 PM **LUNCH/NETWORKING** CCIS PCL Lounge

SESSION III: PREVENTION, LIFESTYLE, AND SURVIVORSHIP CCIS 1-440
Chair: Dr. Vincent Biron, Co-Chair: Dr. Fawaz Makki

12:40 PM – 12:55 PM Dr. Catherine Field, Professor
Department of Agriculture and Nutrition
Harnessing fatty acids to improve breast cancer treatment

12:55 PM – 1:10 PM Dr. Kerry Courneya, Professor and Canada Research Chair in
Physical Activity and Cancer
Department of Physical Education and Recreation
Exercise and cancer outcomes: From observational studies to randomized trials

1:10 PM – 1:25 PM Dr. Serena Rix, Pharm D.
Department of Pharmacy & Pharmaceutical Sciences
Filing the gaps: Identification of Educational Needs in Palliative Care

1:25 PM – 1:40 PM Dr. Priva Sivarajah, Resident
Department of Surgery
Depression as a predictor of postoperative functional performance status (PFPS) and treatment adherence in head and neck cancer patients: a prospective study

1:40 PM – 1:50 PM **SHORT BREAK** CCIS 1-440

SESSION IV: NOVEL THERAPEUTICS AND BIOMARKERS CCIS 1-440

Chair: Dr. Thomas Simmen, Co-Chair: Mohamed Salla

1:50 PM – 2:05 PM Dr. Michael Oveduin, Professor
Department of Biochemistry
Structure and function of novel cancer targets

2:05 PM – 2:20 PM Dr. Richard Fahlman, Professor
Department of Biochemistry
Insights into Tumor Cell Death and Biomarker Identification by Proteome Profile

2:20 PM – 2:35 PM Dr. Michael Chu, Assistant Professor
Medical Oncology
Cross Cancer Institute
Radiographic myosteatorsis is prognostic and predictive of ipilimumab outcomes in melanoma

2:35 AM – 2:50 PM Hashem Etayash, PhD Student
Department of Pharmacy
Nanomechanical Sandwich Assay for multiple Cancer Biomarkers in Breast Cancer Cell-derived Exosomes

2:50 PM – 4:05 PM **POSTER SESSION 2/SNACKS** CCIS PCL Lounge
EVEN NUMBERED POSTERS

SESSION V: THE NEXT GENERATION

CCIS 1-440

Chair: Dr. Khaled Barakat, Co-Chair: Jihane Mriouah

4:05 PM – 4:20 PM Dr. Olena Bilyk, Postdoctoral Fellow
Department of Oncology
Nodal, an embryonic morphogen, promotes cellular plasticity and resistance to therapy in ovarian cancer cells

4:20 PM – 4:35 PM Ashlee Matkin, Medical Student
Department of Surgery
Enhancing the Accuracy of Thyroid Fine Needle Aspirate Biopsies Using Droplet Digital PCR

4:35 PM – 4:45 PM

CLOSING REMARKS AND AWARDS

CCIS 1-440

4:45 PM – 6:30 PM

AWARDS, RECEPTION, & CASH BAR

CCIS PCL Lounge

SPEAKER ABSTRACTS

Pages 11-21

(Poster abstracts are on pages 23-124)

Keynote Address

Dr. Jennifer Chan

**Associate Professor, Departments of Pathology, Oncology, and Clinical Neurosciences
Deputy Director, Charbonneau Cancer Institute
Kids Cancer Care Foundation Chair in Pediatric Oncology Research
University of Calgary**

Title: Oligodendroglioma: from Pattern Recognition to Molecular Mechanisms

Dr. Chan's research program:

Dr. Chan's laboratory research interests stem from the material she sees on the neuropathology service. Her work applies concepts from developmental neurobiology to further understand mechanisms of disease in brain tumorigenesis. She directs the neuro-oncology tumor banking program in Calgary and has launched a tumor banking program at the Alberta Children's Hospital. In the lab, her work examines the intersection of growth factor signaling and intrinsic determinants such as transcription factor function in cell fate determination and proliferation in neural development and cancer. She uses techniques of somatic transgenesis to introduce genes of interest into neural stem and progenitor cells to model tumorigenesis, and supplements this with in vitro studies using brain tumor initiating cells (BTICs) derived from patient material. She is also involved in several multi-institutional projects to molecularly characterize pediatric and adult brain cancers, and is part of a team that aims to bring personalized oncogenomics to childhood cancer patients across Canada.

Dr. David Eisenstat
Faculty of Medicine & Dentistry
Department of Pediatrics
PI

Characterization of Ganglioglioma as a Neurodevelopmental Disorder: A Case of Arrested Development?

Q. Jiang, J. Zagozewski, P. Nozza, B. Wilson, F. Van Landeghem, D.D. Eisenstat

Gangliogliomas (GG) are low grade neuroepithelial tumours of the central nervous system (CNS) comprised of neoplastic glial and neuronal cells. There are no animal models or cell lines to study. Microarray data of a panel of low grade gliomas including GG revealed overexpression of the DLX2 homeobox gene required for tangential interneuronal migration and differentiation in the embryonic forebrain. We hypothesized that DLX2 regulates neural versus glial cell fate decisions in CNS progenitors and that GG are arrested in development. METHODS: DLX2 expression was examined along with glial fibrillary acidic protein (GFAP; glial marker) and synaptophysin and/or NeuN (neuronal markers) expression in a cohort of GG tumours using immunohistochemistry and dual immunofluorescence labelling of formalin fixed paraffin embedded (FFPE) tissue sections. BRAF mutational status was also assessed. RESULTS: In our discovery cohort (Genoa), 10/30 samples (33%) expressed DLX2. In our validation cohort (Edmonton), 22/36 (61%) expressed nuclear DLX2 and 12/22 DLX2+ cases had BRAF mutations (55%; 11 V600E, 1 V600G). 12/18 cases with BRAF mutations were DLX2+ (67%). One heavily pre-treated child with progressive cervicomedullary GG has had a very good partial response to BRAF inhibitor therapy. All 22 DLX2+ tumours co-expressed GFAP (100%) and 21/22 (95%) also expressed synaptophysin or NeuN. CONCLUSIONS: Our results support GG as neurodevelopmental tumours arising from bipotential CNS progenitors that are arrested at the neural-glial cell fate "decision" point. Biological or differentiation-based treatments could be considered +/- BRAF inhibitors for those GG with/without the V600E mutation, respectively.

Saket Jain
Faculty of Medicine & Dentistry
Department of Oncology
Graduate Student

Role of Transcription factor AP-2 in Glioblastoma Pathogenesis

Saket Jain, Rongzong Liu and Roseline Godbout

Glioblastoma (GBM) are highly aggressive brain tumours. Patients diagnosed with GBM have a dismal prognosis, with a median survival of 15 months. Activating Protein 2 (AP-2) is a family of transcription factors (AP-2a, b, c, d and e) involved in the regulation of genes responsible for early development, cellular growth and differentiation. The subcellular localization of AP-2a has been associated with astrocytoma tumor grade. In low grade astrocytoma, AP-2a is primarily found in the nucleus, whereas in GBM, it has a cytoplasmic pattern. Furthermore, our immunofluorescence and nuclear-cytoplasmic fractionation analyses indicate that AP-2b localize to the cytoplasm of GBM cells. We are particularly interested in AP-2b as its expression correlates with poor survival in GBM patients. We are using patient-derived tumor neurospheres as our experimental model. Our data reveals that AP-2b is more highly expressed in neurosphere cultures as compared to adherent cultures derived from the same patients. Interestingly, AP-2b localizes to the nucleus of neurosphere cultures. Knockdown of AP-2b in GBM neurosphere cultures results in decreased expression of stem cell markers SOX2, as well as decreased cell migration. Stem cell maintenance and mesenchymal characteristics are associated with hypoxia in GBM. We observed an increase in AP-2b levels in GBM neurospheres cultured in 0.5% O₂. Our preliminary immunohistochemical analysis of GBM tumor tissue sections show expression of AP-2b in pseudopalisading cells surrounding necrotic regions. These combined data suggest that AP-2b may regulate stem cell maintenance and migration in GBM. Our future objectives are to determine: (i) the mechanisms by which AP-2 affects the growth properties of GBM cells, and (ii) the mechanism by which AP-2 subcellular localization is regulated and how it affects stem cell maintenance in GBM. We will also examine how AP-2 expression affects GBM response to radiation and chemotherapeutic agents such as temozolomide.

Dr. Anahita Mojiri
Faculty of Medicine & Dentistry
Department of Medicine
Postdoctoral Fellow

Functional assessment of Von Willebrand factor (VWF) expression by cancer cells of non-endothelial origin

Anahita Mojiri, Konstantin Stoletov, Lian Willetts, Maria Areli Lorenzana Carrillo, Roseline Godbout, Paul Jurasz, Consolato M. Sergi, David D. Eisenstat, John D. Lewis, and Nadia Jahroudi

Introduction: Von Willebrand Factor (VWF) is an endothelial specific, adhesive, procoagulant molecule. Increased plasma levels of VWF and alterations in coagulation system in cancer patients with metastatic progression are reported. We hypothesized that some cancer cells with non-endothelial origin, express VWF, which facilitates their metastasis.

Methods: Several glioma and osteosarcoma cell types were analyzed for VWF expression using quantitative real time-PCR, western blot and immunofluorescence (IF) staining. Chromatin immunoprecipitation analyses were performed to determine association of VWF promoter with its regulatory transcription factors and its epigenetic modifications, in VWF expressing and non-expressing cancer cells. Adhesion and transmigration ability of VWF-expressing cancer cells were explored using FACS and Transwell assays. Extravasation ability of cancer cells was examined in Chick Chorioallantoic Membrane (CAM) assays and in a metastatic mouse model. Tumor patients' biopsies were analyzed for VWF-expressing cancer cells using IF staining.

Results: De novo expression of VWF, at the RNA and protein levels, was demonstrated in several glioma and osteosarcoma SAOS2 cell lines. The pattern of transacting factors bindings and epigenetic modifications in VWF expressing cancer cells were similar to that in endothelial cells. Cancer cells expressing VWF demonstrated enhanced endothelial-adhesion and transmigration. Extravasation capacities of VWF-expressing cancer cells were significantly enhanced compared to those not expressing VWF. VWF knock down significantly reduced adhesion, transmigration and extravasation of VWF-expressing cancer cells, while over-expression of exogenous VWF in VWF non-expressing cancer cells resulted

in their increased adhesion and extravasation capacities. VWF expressing cancer cells were detected in patient's tumor biopsies of glioma and osteosarcoma.

Conclusion: De novo expression of VWF in cancer cells may enhance their metastatic potential.

Dr. Serena Rix

Pharmacy & Pharmaceutical Sciences

Department of Pharmacy

PI

Filing the gaps: Identification of Educational Needs in Palliative Care

Rix S, Hellec B, Marsh S

Objectives: Research suggests an increased desire for palliative care patients to remain at home as long as possible, resulting in a need for pharmacists to be more involved in the care of this distinct population. We aimed to identify gaps in palliative care education and develop metrics for the areas to target educational strategies. **Methods:** We assessed de-identified discharge records for 75 palliative patients from a Tertiary Palliative Care Unit (TPCU) between 2009 and 2014. We also developed an 8 question survey for community pharmacists to assess their comfort level in providing services to this population. **Results:** From the discharge record assessment we found patients left the hospital with an average of 7.8 regular prescription medications, and 2.7 “as needed” medications. The majority of patients were discharged with opioids (96%) and 87% were also receiving laxatives. Other common medications included corticosteroids, anti-emetics, anxiolytics, anti-depressants and anti-microbials. Some patients were discharged on chemotherapy, anti-coagulants, and agents for co-morbid diseases. Of the 47 community pharmacist survey responses, 42 were complete. Over 50% stated they had no palliative care education. Whilst almost 70% of responders reported being comfortable or very comfortable with the use of opioids for pain control, only 30% of responders were comfortable or very comfortable with the use of corticosteroids for cancer-related problems, and only 26% were comfortable or very comfortable with the use of neuroleptics for issues including delirium. **Conclusions:** Pharmacists need educational materials and support to improve their knowledge of palliative care; this includes safe off-label use, polypharmacy, and providing other caregivers with relevant information. The data collected from this study will drive the content of palliative education for both practising

pharmacists and students. Additionally it will contribute to development of clinical decision support apps to aid practitioners in the delivery of comprehensive at-home care to palliative patients.

Dr. Priya Sivarajah
Faculty of Medicine & Dentistry
Department of Surgery
Resident

Depression as a predictor of postoperative functional performance status (PFPS) and treatment adherence in head and neck cancer patients: a prospective study

Brittany Barber MD, Priya Sivarajah MD, Jace Dergousoff MD, Peggy Nesbitt AP, Nicholas Mitchell MD FRCPC, Jeffrey Harris MD MHA FRCSC, Daniel O'Connell MD MSc, David Cote MD MPH CCFP FRCSC, Vincent Biron MD PhD FRCSC, Hadi Seikaly MD MAL FRCSC

Background: Head and neck cancer (HNC) is a debilitating disease due in part to its effects on speech, swallowing, and cosmesis. The aim of this study was to assess the relationship between preoperative depressive symptoms (PDS) and postoperative functional performance status (PFPS), in addition to other predictors of rehabilitation and survival.

Methods: A prospective cohort study was undertaken at the University of Alberta, including all new adult HNC patients undergoing surgery as primary therapy for HNC from May 2013 to January 2014. Baseline depressive symptoms were measured on the Quick Inventory of Depressive Symptoms (QIDS) questionnaire 2 weeks preoperatively and PFPS was assessed 12 months postoperatively on the Functional Assessment of Cancer Therapy-Head & Neck FACT-HN) scale. Secondary outcomes included completion of adjuvant therapy, narcotic dependence, return to detrimental habits, loss of follow-up, and length of hospital stay (LOHS). Differences between the Normal-Mild and Moderate-Severe QIDS groups were assessed using Mann-Whitney and Fischer Exact statistical analyses. Survival to date was analyzed using Kaplan-Meier analysis.

Results: Seventy-one patients were included in the study. Mild and Moderate Severe PDS were 35.2 % and 18.3 %, respectively. Significantly lower FACT-HN scores were noted in the Moderate-Severe group at 12 months ($p=0.03$). The risk ratio (RR) for FACT-HN score <50 %

at 12 months in the Moderate-Severe group was 5.66. In addition, significantly lower completion of adjuvant treatment ($p=0.03$), significantly higher incidence of narcotic dependence ($p=0.004$), and significantly higher LOHS (24 days vs. 18 days; $p=0.02$) was observed in the Moderate-Severe group. Survival was not significantly different between groups at approximately 18 months ($p=0.960$).

Conclusions: Patients with Moderate-Severe PDS have significantly decreased PFPS, increased narcotic use, decreased completion of adjuvant therapy, and a longer LOHS after surgical treatment for HNC.

Dr. Richard Fahlman
Faculty of Medicine & Dentistry
Department of Biochemistry
PI

Insights into Tumor Cell Death and Biomarker Identification by Proteome Profiling.

Richard P. Fahlman

Advancements in quantitative mass spectrometry have been enabling the quantification of cellular proteomes to levels previously unobtainable. These newer methods enable the quantitative comparisons of whole proteomes for a variety of applications. Here we will summarize two recent investigations on the whole proteome analysis of tumors. The first investigation is focused on the *in vivo* response of EL4 cell line derived mouse tumours to etoposide-cyclophosphamide co-treatment. This data provides new insights into the molecular mechanism of tumor death in this model system, including potential roles for caspase-6 and lysosomes. The second investigation used quantitative proteomics to investigate the proteomes of estrogen receptor positive breast tumours in a study focused on further sub-typing this type of breast cancer to identify indicators of disease proteins relapse. Preliminary data indicates there are a number of proteins identified in these tumours that predict disease recurrence that need to be validated with larger clinical datasets.

Dr. Michael Chu
Faculty of Medicine & Dentistry
Department of Oncology
PI

Radiographic myosteatosi s is prognostic and predictive of ipilimumab outcomes in melanoma

Michael P. Chu, Yuetong Li, Sunita Ghosh, Shelley Sass, John Walker, Michael Smylie, and Michael B. Sawyer

Background Myosteatosi s appears as low radiographic density (called skeletal muscle density, SMD) on computed tomography (CT) imaging. Its presence is prognostic among many cancers. SMD has not been investigated in immunotherapeutic-treated malignant melanoma (MM). This retrospective study examined SMD's prognostic ability in ipilimumab-treated MM patients. Methods Advanced/metastatic MM patients (pts) treated with ipilimumab from 2009-2014 were reviewed. Pre-treatment CT images were used to measure SMD at the third lumbar vertebrae as previously described and expressed in Hounsfield Units (HU). Cutpoint analysis determined whether a particular level of SMD demonstrated differences in progression free (PFS) and overall survival (OS). Secondary endpoints included objective response rates (ORR) and toxicities. Results Of 121 identified, 97 pts were evaluable. Baseline demographics included: 56 years median age, 58 male (60%), and 23 with BRAF mutations (23.7%). Cutpoint analysis identified a difference between pts with SMD < 42 and 20 HU for pts with BMI < 25 and ≥ 25 kg/m², respectively. Pts with low SMD had poorer median PFS (2.4 vs. 2.7 months, hazard ratio [HR] 1.76, p=0.008) and OS (5.4 vs. 17.5 months, HR 2.47, p=0.001) compared to pts with SMD above the cutpoint; these differences remained in multivariate Cox proportional hazards modeling with age, gender, BRAF status, and line of treatment. There was no statistical difference in toxicity between SMD groups, but ORR trended in favor of higher SMD (17.9 vs. 3.3%, p=0.051). SMD may relate to inflammation given a higher prevalence of high neutrophil-to-lymphocyte ratio in low SMD pts (39 vs. 21%, p=0.049). Conclusion Low SMD is prognostic of melanoma outcomes in the immunotherapy era. SMD may relate to an underlying inflammatory state and therefore predict who may or may not respond to such therapy. It may also therefore point toward to immunotherapy resistance mechanisms.

Hashem Etayash
Pharmacy and Pharmaceutical Sciences
Department of Pharmacy
Graduate Student

Nanomechanical Sandwich Assay for multiple Cancer Biomarkers in Breast Cancer Cell-derived Exosomes

Hashem Etayash, A. R. McGeeb, K. Kaur and Thomas Thundat

Exosomes are nanoscale vesicles with sizes in the range of 30 nm – 150 nm shed by many cell types into the bloodstream, including cancer cells. As they harbour a number of bioactive receptors, nucleic acids, and signalling proteins for cell-to-cell communications, they have become increasingly attractive diagnostic and therapeutic targets. The use of exosomes as cancer diagnostic biomarkers; however, is limited by their size, heterogeneity and the need for extensive purification and labelling. In this study we report the use of cantilever sensor arrays for simultaneous detection of multiple exosomal surface-antigens with high sensitivity and selectivity. Exosomes derived from breast cancer cells were selectively identified from a human serum by targeting over-expressed membrane-proteins CD24, CD63, and EGFR. Excellent selectivity; however, was achieved when targeting the cell-surface proteoglycan, glypican-1 (GPC1) at extraordinary limits (~ 200 exosomes ml⁻¹, ~ 0.1 pg ml⁻¹). The study showed further enhanced sensitivity of the detection by using a nanomechanical sandwich assay, where antibodies-coated gold nanoparticles were introduced into the cantilever sensor. This finding is the first to identify GPC1 as a selective biomarker for breast cancer exosomes. It also offers opportunities for the development of exosomes isolation techniques for tracking breast tumors and monitor efficiency of treatments.

Dr. Olena Bilyk
Faculty of Medicine and Dentistry
Department of Oncology
Postdoctoral Fellow

Nodal, an embryonic morphogen, promotes cellular plasticity and resistance to therapy in ovarian cancer cells

Olena Bilyk, Jiahui Liu, Scott Findlay, Lynne-Marie Postovit

Introduction: Following chemotherapy residual cancer cells could employ embryonic stem cell signaling pathways to sustain plasticity and resistance to therapy. Nodal is a potent embryonic morphogen which has been found to sustain stem cell pluripotency and cellular plasticity. However, Nodal expression re-emerges in cancer promoting cancer stem cell renewal, tumor growth and metastasis. We hypothesize that Nodal signaling drives plasticity in OC cells and that disease progression and therapy resistance will be mitigated when Nodal is inhibited.

Methods: We applied in vitro assays designed to assess growth (MTT and clonogenic assays), stem cell like phenotypes (sphere limiting diluting assays) and chemoresistance (surviving fraction assays and a human cancer drug resistance PCR array) in OC cells (A2780s) wherein Nodal was added with rhNodal or a Nodal expression construct, or knocked down/out with shRNA or CRISP/Cas9 genome editing.

Results: We found that Nodal is significantly up-regulated in OC cells (A2780s) in response to platinum drug treatment. When Nodal was ectopically expressed in OC cells, it promoted partial epithelial-to-mesenchymal transition (cellular plasticity) and increased resistance to carboplatin while Nodal knockdown sensitized cells to drug. Moreover, Applying of rhNodal prevented cell cycle arrest in OC cells after treatment with platinum. Drug resistance gene expression array identified the up-regulation of 21 genes in Nodal expressing cells as compared to controls. ERBB4 was the most upregulated.

Conclusion: These data demonstrate that Nodal is likely driving cancer cell plasticity and resistance chemotherapy in OC cells by promoting cancer stem cell-like phenotype and by upregulating target genes involved in multi-drug resistance, and may hold promise as a therapeutic target to prevent OC recurrence.

Ashlee Matkin
Medicine and Dentistry
Department of Surgery
Medical Student

Enhancing the Accuracy of Thyroid Fine Needle Aspirate Biopsies Using Droplet Digital PCR

Ashlee Matkin, Morris Kostiuik, Karina Currie, Daniel A. O'Connell, Hadi Seikaly, David W.J. Cote, Jeffrey Harris, and Vincent L. Biron

Approximately 25% of cytology reports from thyroid nodule fine needle aspiration biopsies (FNABs) are classified as indeterminate. Many patients with this result will undergo a diagnostic hemithyroidectomy, with up to 70% of cases found to be benign on final pathology. Enhancing the accuracy of FNABs could therefore reduce unnecessary thyroid surgery. Droplet digital PCR (ddPCR) has the potential to identify pathogenic mutations and aberrant gene expression indicative of thyroid carcinoma using a low concentration of nucleic acid. This study aims to determine if ddPCR is a reliable method of biomarker analysis in thyroid FNABs. Ultrasound-guided FNABs were prospectively collected from 102 patients presenting with thyroid nodules at a tertiary referral centre for head and neck surgery. Samples were stored in RNA-preserving reagent for later nucleic acid extraction. ddPCR was performed on samples using ≥ 1 ng of cDNA with the following HEX/FAM-labelled probes. Gene expression levels were measured relative to an internal control (EEF) for PTEN, PIK3CA, MET, CCND1, MK167, TSHR, PPAR-g, LGALS3 and EGFR. Mutational analysis was performed with comparison to wild type control for HRAS pG12V, HRAS Q61R, HRAS Q61K, NRAS Q61R, NRAS Q61K and BRAF V600E. The primary outcome measure compared genetic profiling results to standard cytology reports and final histopathology in patients who received surgery. Mutational and gene expression data was reliably obtained with small amounts of nucleic acid within 24 hours of receiving the sample. Mutations were identified in a number of FNAB specimen, including the samples that resulted in a diagnosis of malignancy. Gene expression profiles demonstrated a broad range of expression for all genes tested. The detection of biomarkers of thyroid cancer in FNABs, through the use of ddPCR, may enhance the accuracy of FNABS and has the potential to become a new diagnostic tool.

POSTER ABSTRACTS

Pages 23-124

(Speaker abstracts are on pages 11-21)

Poster Presentations

1

Danny Aceytuno

Medicine

Biochemistry

A severe mutation from the neurological disease MCSZ dysregulates the DNA damage response and repair by the Polynucleotide Kinase/Phosphatase-XRCC4-DNA Ligase IV NHEJ end-processing complex

R. Daniel Aceytuno, Cortt G. Piatt, Zahra Haval-Shahriari, Ross A. Edwards, Martial Rey, Shujuan Fang, Michael Weinfeld, Michal Hammel, John A. Tainer, David C. Schriemer, Susan P. Lees-Miller, and J. N. Mark Glover

If unrepaired or misrepaired, DNA double-strand breaks (DSBs) can lead to genomic instability and cell death or neoplastic transformation. The major DSB repair (DSBR) mechanism in higher eukaryotes is non-homologous end-joining (NHEJ). In NHEJ, polynucleotide kinase/phosphatase (PNKP) is the primary enzyme for processing abnormal 5'-hydroxyl and 3'-phosphate ends that prevent the final repair step by XRCC4/DNA Ligase IV (Lig IV). This processing step is thought to be mediated by an interaction between the PNKP-FHA domain and CK2-phosphorylated XRCC4 C-terminal tails. However, our results from binding experiments show tight binding between XRCC4/Lig IV and PNKP both with and without CK2-phosphorylation of XRCC4. We determined a low-resolution ensemble structure of the purified phosphorylated-XRCC4/Lig IV/PNKP ternary complex by small-angle X-ray scattering (SAXS) experiments, which suggests a second phosphorylation-independent interaction between the PNKP and XRCC4/Lig IV. We used hydrogen-deuterium exchange (HDX) experiments to probe potential PNKP secondary interaction sites with XRCC4/Lig IV and identified a candidate interaction site within a loop in the PNKP phosphatase domain. This site contains the clinically significant PNKP E326K mutation found in the severe form of the hereditary neurological disease MCSZ (microcephaly with early-onset intractable seizures and developmental delay). Activity assays show that the E326K mutation disrupts both substrate binding and turnover in PNKP when bound to phosphorylated-XRCC4/Lig IV. Furthermore, our UV laser microirradiation experiments in cells show that the E326K mutation also disrupts recruitment of PNKP to DNA lesions. We have identified a putative secondary interaction site that functionally contributes both to recruitment and catalysis of PNKP in DNA strand break

Poster Presentations

repair. Disruptions to PNKP in this region may result in decreased DNA double-strand break repair in cells and describe a molecular basis of MCSZ. This interaction surface may also prove an interesting target for small-molecule inhibition of NHEJ toward novel radio- and chemo-sensitizing therapies in cancer treatment.

2

Helya Aghazadeh

Medicine and Dentistry

Ophthalmology

A comparison of the association of ultraviolet light exposure with cutaneous and uveal melanoma: a meta-analysis.

Aghazadeh H., Weis E.

Uveal melanoma (UM) is the single most common primary ocular malignancy. Although several host susceptibility factors have been shown to be associated with UM, a clear relationship with sun exposure has not been demonstrated a significant association utilizing a meta-analytic approach by Weis et al. In contrast, a meta-analysis conducted by Gandini et al. investigating the relationship between sun exposure and cutaneous melanoma concluded that intermittent sun exposure and sunburn history are positively correlated with the risk of developing cutaneous melanoma, whereas high occupational sun exposure was shown to have an inverse correlational relationship.⁵ The establishment of this relationship has led to highly effective prevention programs and presents the potential of developing more targeted therapies. As a result, our study aims to compare these two bodies of literature in the form of a cumulative meta-analysis, allowing us to determine whether small sample size issues are resulting in the absence of a statistically significant association between UV exposure and uveal melanoma. Understanding where uveal melanoma research currently stands will allow researchers to more effectively investigate the relationship between UV light exposure and the development of uveal melanoma. Furthermore, a greater appreciation of this relationship will facilitate a better understanding of the etiology of uveal melanoma, potentially facilitating the development of an effective prevention and treatment options for patients.

Sadra Aghazadeh

Faculty of Science

Department of Biological Sciences

Expression of BFABP in the progression and differentiation of glioblastoma

Sadra Aghazadeh, Epsita Shome, Ryan Fung, Roseline Godbout, David D. Eisenstat

Introduction: Glioblastoma (GBM) is the most common malignant brain tumour and is among the most aggressive forms of cancer with less than 10% survival at 5 years. To date, only one prognostic biomarker has been identified for GBM patients, MGMT promoter methylation.

This project builds on prior work from our lab studying cell death genes and patient outcomes in GBM as well as Dr. Godbout's lab which has focused on a protein called brain fatty acid binding protein (BFABP). My focus is to determine whether BFABP, also known as FABP7, which is expressed in radial glia, influences the progression of pediatric and adult GBM tumor cells as determined by proliferation and migration. We expect to gain insight into FABP7 as a potential prognostic biomarker for GBM. Methods: Immunohistochemistry was used to study FABP7 expression on a cohort of 81 formalin-fixed paraffin-embedded (FFPE) GBM patient samples treated with surgery and chemoradiation with adjuvant cis-retinoic acid and both concurrent and adjuvant temozolomide. Expression patterns will be scored and correlation with patient outcomes determined. Immunostained patient samples will also be used to compare the expression of FABP7 with GFAP and developmental markers, such as DLX2, PAX6 and PROX1. Lastly, siRNA or shRNA transfection methods will be used to assess how knockdown of FABP7 affects GBM cell lines that express high endogenous levels of FABP7 in vitro (U251, U373). Flow cytometry will be used to assess cell cycle markers, whereas cell death and invasion assays (ex. Boyden chamber) will be used to evaluate the effects on tumor cell migration in vitro. Results: Analysis of this data is currently underway but will help determine if FABP7 is a potential biomarker for the progression of GBM in patients and whether modulation of its expression influences the invasive properties of malignant glial cells.

Marawan Ahmed

Pharmacy

Pharmaceutical sciences

Rational Design and Validation of Of Small Molecular Inhibitors For The PD-1/PD-L1
Immune-Checkpoint Pathway

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Houghton^{2,3,6} and Khaled Barakat^{1,3,6}*

Blocking the PD-1/PD-L1 pathway recently emerged as a ‘game changing’ in cancer immunotherapy and monoclonal-antibodies (MABs) targeting PD-1 has been selected as ‘drug of the year’ for 2013 [1]. Although these antibodies restored exhausted T cells’ function to recognize and kill tumor cells, these MABs have numerous disadvantages. This includes their very high cost and very severe side effects [2]. Our team has been focused on designing small molecule inhibitors for this pathway. Compared to available MAB therapies, our small molecule can offer a more affordable, more easily administered, more easily controlled treatment that could treat a variety of cancers including advanced solid tumors and brain tumors. Here, we summarize our efforts toward this goal and summarize preliminary data on compound #HEKA111, a small molecule inhibitor for the PD-1/PD-L1 pathway that binds to PD-1 and restores the proliferative capacity of exhausted T cell.

Mahsa Alaei

Medicine and dentistry

Oncology

Anti-tumor/metastasis activity of plakoglobin in invasive ovarian carcinoma cells

Mahsa Alaei, Manijeh Pasdar

Ovarian cancer (OVCA) is the leading cause of all female reproductive cancer deaths worldwide, with the overall five-year survival rate of ~ 45%. Epithelial cells are held together by adhesion complexes containing E-cadherin, beta-catenin and plakoglobin (PG, gamma-catenin). Disruption of cadherin-catenin complexes is a major contributing factor to both tumor development and progression. Furthermore, upon dissociation from E-cadherin, catenins can interact with other cellular proteins and regulate pathways involved in tumorigenesis/metastasis. In this context, beta-catenin has oncogenic function, whereas PG acts as a tumor/metastasis suppressor (TMS). The oncogenic activation of beta-catenin is well documented in OVCA. However, while the loss of heterozygosity of the PG gene has been reported in OVCA, little is known about how it acts as a TMS in this neoplasm. Here, we have investigated the role of PG in OVCA. We have shown that one mechanism by which PG acts as a TMS is by interacting with p53, a TS that is mutated in >50% of OVCA. We also have shown that PG interacts with both WT and mutant p53s (mp53s) and its interaction with mp53s restores their TS activities. We assessed the in vitro TMS effects of PG in epithelial OVCA cell lines with mp53 expression and different adhesion profiles. We showed that PG-deficient OVCA cells that express N-cadherin and mp53 were highly migratory and invasive, whereas those that express mp53 and PG were not. The exogenous expression of PG or knockdown of N-cadherin significantly reduced migration and invasion. PG colocalized with cadherins in adhesion complexes and with WT and mp53. Consistent with these observations, we detected significant reduction in growth, migration and invasion of PG expressing and N-cadherin knockdown cells. Our data suggest that PG can induce growth/ metastasis inhibitory effects in OVCA cells expressing N-cadherin and mp53.

Abdulrahman Alenazi
Medicine and Dentistry
Surgery

Biomarker Profiles of Oral Cavity Squamous Cell Carcinoma Correlated with Patient Survival

Abdulrahman Alenazi, Lakshmi Puttagunta, Morris Kostiuk, Daniel A. O'Connell, Jeffrey Harris and Hadi Seikaly and Vincent L. Biron

Objectives: Over 300,000 new cases of oral cavity squamous cell carcinoma (OCSCC) are diagnosed yearly worldwide. OCSCC is molecularly heterogeneous, which is thought to contribute to differences in treatment response between patients who have otherwise similar characteristics. The objective of this study is to examine the role of a combination of important tumor biomarkers in predicting outcomes of patients with OCSCC. Materials and Methods: Patient demographics, pathology and treatment information for diagnosed with advanced stage OCSCC between 1998-2010 was obtained from a provincial cancer registry. A tissue microarray was constructed and processed for immunohistochemistry with p16, p53, Bcl-XL, EGFR and Ki-67 antibodies. Additional staining with pancytokeratin and DAPI was used for 3-channel co-localization and quantification of biomarkers in normal vs tumor tissues. Biomarker expression levels were correlated with tumor recurrence, metastases and patient survival. Results: Between 1998-2010, 584 patients were diagnosed and treated for OCSCC at a single tertiary care center. Nearly 70 % of these patients presented with advanced stage disease and were retrospectively reviewed for biomarker analysis. P16 positivity was found in 16.6 % of these tumors but was not predictive of survival. Low levels of Ki-67 was associated with lower survival rates and poorer treatment responses to radiation. Combined EGFR and Ki-67 ratios were also associated with significant differences in survival. Conclusions: Biomarkers analysis in advanced stage OCSCC including Ki67 and EGFR may be predictive of patient outcomes. Further prospective studies should be undertaken to examine the role of these biomarkers in selecting optimal treatment regimens.

Noureen Ali

Faculty of Medicine and Dentistry

Pediatrics

Role of Beta-catenin/Active Beta-catenin in Osteosarcoma progression

Noureen Ali, Geetha Venkateswaran, Elizabeth Garcia, Sujata Persad

Background: Osteosarcoma (OS) is the most common primary bone malignancy with high incidence in children and adolescents, with the lowest overall survival rate. The Wnt signaling pathway, deregulated in most cancers, has been implicated to be deregulated in OS. However, the role of Beta-catenin (key regulatory component of Wnt signaling) in OS is not clear. Further, there is no report on the role of Active Beta Catenin (ABC), the fraction of Beta-catenin that is transcriptionally active, in OS. ABC transcribes genes involved in cell proliferation, invasiveness and hence promotes cancer. Therefore in our study we are interested in investigating the role of Beta-catenin/ABC in OS progression. Methods: We used two pairs of cell lines that represent OS progression: Saos2, Saos2-LM7 (metastatic cell line derived from SaOS2), HOS and HOS-143B (metastatic cell line derived from HOS). OS specific markers like MMP2 and MMP9 were used as a measure of aggressiveness in cell lines. Western blotting, Immuno-fluorescence and high content analysis were carried out for determining the cellular localization of Beta-catenin and ABC. Quantitative-RT-PCR was also carried out to measure gene expression levels of downstream targets of Beta-catenin/ABC including MMP2, MMP9, Cyclin D1 and VEGFA. Results: We saw higher cellular levels of ABC in both metastatic cell lines compared to their respective parental cell lines. In addition, we observed higher nuclear localization/levels of ABC in the metastatic cell lines compared to parental. However, no significant changes were observed in the cellular/nuclear localization of Beta-catenin with OS progression. Further, with the exception of MMP9 in HOS/ HOS-143B, we observed a significantly higher expression of all measured genes in the metastatic cell lines compared to parental. Conclusion: Our results show a correlation between ABC levels and OS progression, demonstrating its potential to serve as a prognostic marker for OS progression.

Zaid Almaayah

Pharmacy and Pharmaceutical sciences

Pharmaceutical sciences

Mechanistically Elucidating the In-Vitro Safety and Efficacy of a Novel Doxorubicin

Derivative

Zaid H Maayah¹, Samaa Alrushaid², Casey L. Sayre³, Yunqi Zhao⁴, M Laird Forrest⁵, Sanjeewa Senadheera⁵, Kevin Chaboyer^{1,6}, Hope D Anderson^{1,6}, Ayman El-Kadi³, Neal M. Davies^{1,3}

Purpose: Doxorubicin is an effective anticancer drug, however it is cardiotoxic and has poor oral bioavailability. Quercetin is a flavonoid found in plant based food with antioxidant properties, P-glycoprotein (P-gp) and CYP3A4 inhibitory effects. To mitigate doxorubicin's therapeutic barriers, DoxQ, a novel derivative of doxorubicin, was synthesized by conjugating quercetin to doxorubicin. In this study, the in-vitro safety and efficacy of DoxQ are mechanistically characterized. Methods: The drug release in-vitro and the cellular uptake by Multi-drug resistant canine kidney cells (MDCK-MDR) were quantified by HPLC. The antioxidant activity, CYP450 inhibition, and P-gp inhibition effects were examined using commercial assay kits. The drug potency was assessed utilizing triple negative murine breast cancer cells and the cardiotoxicity was examined in both adult rat and human cardiomyocytes (RL-14). Dichlorofluorescein (DCF) assay was used to determine the level of reactive oxygen species (ROS) in RL-14 cells. RT-PCR was used to examine the expression of cardiotoxicity markers, oxidative stress markers, and CYP450 enzymes in RL-14 cells. Results: DoxQ showed lower cytotoxicity to both rat and human cardiomyocytes and also lower levels of ROS and oxidative stress markers compared to doxorubicin. DoxQ inhibited both the expression and catalytic activity of CYP1B1. Additionally, DoxQ inhibited CYP3A4 and exhibited higher cellular uptake by MDCK-MDR cells than doxorubicin. Conclusions: DoxQ demonstrates a novel therapeutic approach to attenuate cardiotoxicity and poor bioavailability of doxorubicin. The cardioprotective mechanism of DoxQ likely involves scavenging ROS and CYP1B1 inhibition. DoxQ may potentially enhance the poor oral bioavailability of doxorubicin by inhibiting CYP3A4 and P-gp.

Abdulraheem Alshareef
Medicine and Dentistry
Lab medicine and pathology

The use of cellular thermal shift assay (CETSA) to study Crizotinib resistance in ALK-expressing human cancers

Abdulraheem Alshareef, Hai-Feng Zhang, Yung-Hsing Huang, Chengsheng Wu, Jing Dong Zhang, Peng Wang, Ahmed El-Sehemy, Mohamed Fares & Raymond Lai

Various forms of oncogenic ALK proteins have been identified in various types of human cancers. While Crizotinib, an ALK inhibitor, has been found to be therapeutically useful against a subset of ALK(+) tumours, clinical resistance to this drug has been well recognized and the mechanism of this phenomenon is incompletely understood. Using the cellular thermal shift assay (CETSA), we measured the Crizotinib-ALK binding in a panel of ALK(+) cell lines, and correlated the findings with the ALK structure and its interactions with specific binding proteins. The Crizotinib IC₅₀ significantly correlated with Crizotinib-ALK binding. The suboptimal Crizotinib-ALK binding in Crizotinib-resistant cells is not due to the cell-specific environment, since transfection of NPM-ALK into these cells revealed substantial Crizotinib-NPM-ALK binding. Interestingly, we found that the resistant cells expressed higher protein level of Beta-catenin and siRNA knockdown restored Crizotinib-ALK binding (correlated with a significant lowering of IC₅₀). Computational analysis of the crystal structures suggests that Beta-catenin exerts steric hindrance to the Crizotinib-ALK binding. In conclusion, the Crizotinib-ALK binding measurable by CETSA is useful in predicting Crizotinib sensitivity, and Crizotinib-ALK binding is in turn dictated by the structure of ALK and some of its binding partners.

Scott Anderson

Medicine and Dentistry

Surgery: Otolaryngology - Head and Neck Surgery

Practices Regarding Human Papillomavirus Counseling and Vaccination in Head and Neck Cancer: A Canadian Physician Questionnaire

Scott Anderson, Andre Isaac, Caroline C Jeffery, Joan R Robinson, Daniela Migliarese Isaac, Christina Korownyk, Vincent L Biron, Hadi Seikaly

Background: Human papillomavirus (HPV) has recently been implicated as a causative agent in a rapidly growing number of oropharyngeal cancers. Emerging literature supports the hypothesis that HPV vaccination may protect against HPV-related head and neck cancer (HNC) in addition to HPV-related cervical and anogenital disease. While the association between HPV infection and cervical cancer is widely understood, its relation to HNC is discussed much less frequently. Objective: To survey HPV counseling practices for infection and vaccination in relation to HNC of primary care physicians (PCPs),

Obstetricians/Gynecologists (OBGYNs), and Otolaryngology - Head and Neck Surgeons (OHNSs) in Canada. Methods: A Canada-wide electronic questionnaire regarding counseling practices on HPV infection, transmission, and vaccination was designed. It was distributed to PCPs, OBGYNs, and OHNSs across Canada through electronic and paper-based methods.

Results: 337 physicians responded (239 family physicians, 51 OHNSs, 30 OBGYNs, and 17 pediatricians). Three out of four PCPs reported routine counseling of their patients regarding HPV infection, transmission, and vaccination. Among this group, 68% reported “never” or “rarely” counseling patients that a serotype of HPV can cause HNC. The most commonly reported reason that PCPs cited for not counseling was a lack of knowledge. The majority of OHNSs (81%) and OBGYNs (97%) counseled patients regarding HPV infection, transmission, and vaccination. However, very few OHNSs (10%) regularly counseled patients with HPV-related HNC about HPV-related anogenital cancer. Similarly very few OBGYNs (18%) regularly counseled patients with HPV related cervical/anogenital cancer about HPV-related HNC. Conclusions: The rate of counseling on HPV infection, transmission, and vaccination in relation to HNC among PCPs is low. The most common reason is a lack of knowledge.

Specialists rarely counsel patients with confirmed HPV-related cancer about other HPV-

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related malignancies. More research is needed on the association between different HPV-related cancers in order to better inform counseling practices.

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Colin Andrews

Faculty of Medicine & Dentistry

Otolaryngology - Head and Neck Surgery

Epidemiology and Survival Outcomes of First Nations Patients with Oropharyngeal Squamous Cell Carcinoma

Andrews, C; Biron, V; Erickson, B; Zhang, H, Makki, F; O'Connell, D; Seikaly, H; Cote, D

Abstract **INTRODUCTION** Oropharyngeal squamous cell carcinoma (OPSCC) is an aggressive cancer that has contributing factors of smoking, alcohol, and human papillomavirus (HPV). The incidence of OPSCC is increasing, despite decreasing smoking rates, due to oncogenic HPV infection. HPV-related OPSCC is associated with favorable survival outcomes. However, there is a paucity of data examining these changing etiological factors and survival outcomes in Canadian First Nations (FN) patients, a population known to have poorer cancer survival and health-related quality of life. **METHODS** This retrospective study used demographic, survival, staging and pathologic data obtained from the Alberta Cancer Registry for patients diagnosed with OPSCC from 1998-2010. This data was cross-referenced to the Alberta Health and Wellness Registry to identify patients with FN status. Clinicopathological differences were compared between FN and non-FN patients. Overall and disease-specific survival was calculated using Kaplan-Meier and Cox regression analyses. **RESULTS** This study identified 345 patients with OPSCC in Alberta of which 15 (4.3%) were First Nations. Analysis of the data demonstrates lower incidence of OPSCC in Alberta FN but no significant differences between FN and non-FN patients in terms of demographics at time of diagnosis.. FN patients were more likely to receive non-surgical treatment. There were no significant differences in 5-year overall and disease-specific survival. **CONCLUSIONS** The incidence of OPSCC in FN is lower than in non-FN people in Alberta. Survival outcomes in FN and non-FN patients with OPSCC are similar. Interestingly, despite similar demographics and stage at presentation, FN patients were more likely to receive non-surgical treatment. These patients receiving non-surgical treatments had lower survival outcomes.

Olena Bilyk
medicine and dentistry
oncology

Nodal, an embryonic morphogen, promotes cellular plasticity and resistance to therapy in ovarian cancer cells

Olena Bilyk, Jiahui Liu, Scott Findlay, Lynne-Marie Postovit

Introduction. Following chemotherapy residual cancer cells could employ embryonic stem cell signaling pathways to sustain plasticity and resistance to therapy. Nodal is a potent embryonic morphogen which has been found to sustain stem cell pluripotency and cellular plasticity.

However, Nodal expression re-emerges in cancer promoting cancer stem cell renewal, tumor growth and metastasis. We hypothesize that Nodal signaling drives plasticity in OC cells and that disease progression and therapy resistance will be mitigated when Nodal is inhibited.

Methods. We applied in vitro assays designed to assess growth (MTT and clonogenic assays), stem cell like phenotypes (sphere limiting diluting assays) and chemoresistance (surviving fraction assays and a human cancer drug resistance PCR array) in OC cells (A2780s) wherein Nodal was added with rhNodal or a Nodal expression construct, or knocked down/out with shRNA or CRISP/Cas9 genome editing. Results. We found that Nodal is significantly up-regulated in OC cells (A2780s) in response to platinum drug treatment. When Nodal was ectopically expressed in OC cells, it promoted partial epithelial-to-mesenchymal transition (cellular plasticity) and increased resistance to carboplatin while Nodal knockdown sensitized cells to drug. Moreover, Applying of rhNodal prevented cell cycle arrest in OC cells after treatment with platinum. Drug resistance gene expression array identified the up-regulation of 21 genes in Nodal expressing cells as compared to controls. ERBB4 was the most upregulated.

Conclusion: These data demonstrate that Nodal is likely driving cancer cell plasticity and resistance chemotherapy in OC cells by promoting cancer stem cell-like phenotype and by upregulating target genes involved in multi-drug resistance, and may hold promise as a therapeutic target to prevent OC recurrence.

Barbara Blasiak

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Dual action core/shell nanoparticles as specific contrast agents

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Magnetic Resonance Imaging (MRI) has been used for early cancer detection, treatment monitoring and image guided surgery. MRI has excellent spatial resolution but low specificity. Standard contrast enhanced MRI, including application of Gd-based T₁ contrast agents do not provide sufficiently high specificity for tumor diagnosis and thus contrast agents providing T₂ contrast have been applied to provide information on tumor specificity (1,2). In particular we are developing core/shell NaDyF₄/NaGdF₄ nanoparticles that may be conjugated with the tumor specific antibodies (2,3). The relaxation times (T₁ and T₂) of the nanoparticles with various core/shell sizes and concentrations were measured at 9.4T and 3T to find the optimum T₁/T₂ ratio for MRI. T₁-, T₂-weighted images of animal models of brain and breast cancer were collected and combined to provide enhanced contrast. The agents consisting of the nanoparticles with the optimal core and shell sizes are being developed to provide improved tumor contrast when the T₁ and T₂-weighted MR pulse sequences are applied. The results may improve the efficacy of the new contrast agents, thus potential suitability for the early detection of cancerous tissues.

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Zackariah Breckenridge

Public Health

Health Policy

Cellular immunotherapies: Lessons in clinical translation

Z. Breckenridge, J. Hutchinson, T. Bubela

Global investments in immunotherapy research recognize its transformative potential to treat cancer. Immunotherapies will add to our anti-cancer arsenal but their promise is based on translational strategies for rational combination therapies in addition to therapies used alone.

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Such combinatorial strategies require advances in structuring collaborative, multidisciplinary, academic-industry teams to move discoveries into the clinic. Developing such strategies involves more than scientific rationales, but knowledge of whether collaborations are best suited to the task compared to market-based licensing and contracting transactions. Based on our comprehensive clinical trials dataset of nearly 1600 cellular immunotherapy clinical trials, we will present an analysis of combinatorial trials, including: therapies, conditions, targets, clinical trial design, sponsors, background intellectual property, licensing/collaboration agreements, financing and any clinical trial outcomes. Our goal is to inform the evolution of collaborative trends in clinical development for cellular immunotherapies.

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Miranda Brun

Agricultural, Life and Environmental Sciences

Agricultural, Food and Nutritional Science

Docosahexaenoic acid reduces tumour growth in combination with docetaxel in a mouse mammary tumour model

M. Brun, M. Newell, C.J. Field

Breast cancer is the most common cancer among Canadian women, accounting for over 25% of diagnoses. Despite ongoing advances in screening, prevention, diagnosis, and treatment, breast cancer remains the second leading cause of cancer-related death in women. Improving treatment, while minimizing side-effects is essential to improving patient outcome. The anti-cancer effects of the long chain omega-3 fatty acids, including docosahexaenoic acid (DHA), found in fish oils have been shown in breast cancer and other cancers. Studies also suggest that DHA can improve the effectiveness of chemotherapy drugs. Docetaxel is a cytotoxic drug used in breast cancer treatment that stabilizes microtubule assembly, suppressing microtubule dynamics leading to inhibition of mitosis. To determine if feeding DHA improves response to docetaxel, nu/nu mice were implanted orthotopically with MDA-MB-231 human breast cancer cells, then mice with established tumours were randomized to high fat diet (20% w/w) \pm 5% w/w DHA, and treated with docetaxel or placebo. Mice treated with DHA+docetaxel had significantly smaller tumours than mice treated with docetaxel alone ($P < 0.05$).

Immunohistochemistry and protein analysis of tumours reveal increased apoptosis and decreased proliferation following treatment with DHA+docetaxel, similar to results examining

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DHA in combination with other chemotherapeutic drugs. This work confirms the beneficial effect of DHA in a commonly used drug for triple negative breast cancer treatment.

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Amirali Bukhari

Medicine and Dentistry

Oncology

Investigating the Role of ATR Inhibition in Improving the Outcome of Radiation Therapy for Breast Cancer

Amirali B. Bukhari, Armin Gamper

Breast cancer remains the second leading cause of cancer-related deaths amongst women in Canada. Around two thirds of cancer patients receive radiation therapy (RT), alone or in combination with chemotherapy. Recent advances in RT, such as image-based planning and directed delivery, have led to enhanced clinical outcomes. Unfortunately, local recurrence and metastasis remain a great problem due to failed eradication of tumor initiating cells. This subpopulation of cancer cells, also termed cancer stem cells (CSCs), has been reported to have a higher self-renewal potential, increased DNA repair capacity, greater metastatic potential, and also increased radioresistance compared to bulk cancer cells. Following ionizing radiation (IR), DNA damage is signaled by two apical kinases, ATM and ATR, of partially overlapping pathways. Radiosensitizers are drugs that IR-induced tumor cell killing with a promise to improve the efficacy of RT. ATR inhibitors have been found to radiosensitize cancer cells, to increase shrinkage of irradiated subcutaneous tumors in mice, and now are in phase I clinical trials. The main aim of our study is to investigate how sensitization of CSCs by a bioavailable ATR inhibitor (AZD6738) can improve the outcome of radiation therapy. Our preliminary studies indicate that inhibition of ATR leads to the downregulation of its downstream target pCHK1 which in turn aids in increased clonogenic cell killing. Furthermore, our initial in vivo studies using the MDA-MB-231-fluc2-tdT orthotopic breast tumor model suggest abundant immunohistochemical staining for gamma-H2AX when NSG mice were treated with a combination of AZD6738 and five fractions of IR (4Gy) using the small animal radiation research platform (SARRP). The long term goal of our combination treatment study is not only to understand the impact on local tumor control, but also metastasis. Since this AZD6738

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has recently entered clinical trials, the outcome of our study can be directly translated to benefit patients.

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Jessica Clark

Medicine and Dentistry

Surgery - Otolaryngology - Head and Neck Surgery

Characteristics of nodal metastases in human papillomavirus positive and negative oropharyngeal squamous cell carcinomas

Jessica Clark, Han Zhang, Scott Murray, Peter Dziegielewski, Vincent Biron, Daniel O'Connell, Jeffrey Harris, Hadi Seikaly

Importance: Oropharyngeal squamous cell carcinoma (OPSCC) is composed of two distinct disease entities. Those cancers related to the oncogenic human papillomavirus (HPV) have been shown to be very different from traditional OPSCC associated with tobacco and alcohol consumption. These diseases not only differ in epidemiological profiles and treatment responses, but also in aggressiveness of local-regional tissue invasion. Despite this, little is known of the patterns and extent of neck metastases for HPV-related OPSCC. Objective: To compare patterns of neck metastasis and extensiveness of neurovascular invasion in HPV-positive and negative OPSCC. Participants: We identified consecutive patients undergoing surgical resection and neck dissection for OPSCC from a prospectively collected database. Univariate analysis was used to identify factors predictive of neurovascular injuries associated with neck dissections and patterns of neck metastasis in the cervical lymph nodes. Results: Two hundred patients were included and 76 patients were found to have HPV-positive disease. Age, sex and tumor stage at initial presentation were similar between the two groups. There were higher rates of N0 disease in HPV-negative cancers with higher rates of advanced nodal disease in HPV-related cancers ($p < 0.05$). Advanced tumor and nodal stage, and ipsilateral nodal disease were associated with contralateral neck metastasis ($p < 0.05$). HPV status was not a statistically significant predictor of contralateral disease; however, we did find that greater than 20% of patients with HPV-related malignancies had contralateral level II involvement. HPV-negative OPSCC was associated with statistically significantly higher rates of ipsilateral spinal accessory, marginal mandibular nerve erosion as well as ipsilateral internal jugular vein injury. Conclusions and Relevance: This is the first study to date to contrast the patterns

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and aggressiveness of HPV-positive and negative OPSCC. HPV-related malignancies had higher rates of nodal disease; however, HPV-negative cancers had higher rates of neurovascular invasion secondary to nodal metastases.

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Dustin Conrad

Dustin Conrad

Otolaryngology Head and Neck Surgery

Metabolic Tumor Volume in Oropharyngeal Squamous Cell Carcinoma, Providing Prognostic Value in a Surgical Cohort

Dustin Conrad, Timothy Cooper, Han Zhang, Hadi Seikaly, Jessica Clark, Caroline Jeffery, Jon Abele, Vincent L. Biron, Jeffrey Harris, Daniel A. O'Connell

Background: Metabolic Tumor Volume (MTV) is a measurement derived from use of 18F-fluorodeoxyglucose (FDG) positron emission tomography with computed tomography (PET-CT). It has been shown as an independent predictive value for survival in patients with oropharyngeal cancer treated with chemoradiation alone. MTV measures any FDG avid area on pre-treatment PETCT meeting the cutoff criteria (SUVmax 2.5). This includes the primary tumor site plus locoregional disease, these values are then summed to give the final MTV value. MTV has previously shown to be an independent prognostic factor in oral cavity squamous cell carcinoma (OPSCC), treated with primary surgery. However, there limited data exploring MTV in relation to oral pharynx squamous cell carcinoma. Objective: To examine OPSCC patients MTV values and determine the prognostic significance of MTV in OPSCC patients treated with primary surgery. Methods: This is a population-based cohort study of patients diagnosed with OPSCC and treated with primary sur

Powel Crosley
Medicine & Dentistry
Oncology

PAC-1 combination with TRAIL enhances apoptosis in cell-line and primary cultured adult granulosa cell tumour cells

Powel Crosley; Kate Agopsowicz; Marjut Pihlajoki; Markku Heikinheimo; Anniina Färkkilä; Mary Hitt

Introduction: Granulosa cell tumour (GCT) constitutes ~5% of ovarian neoplasms and generally responds poorly to chemotherapy. Procaspase activating compound-1 (PAC1) is a small-molecule drug shown in vitro to sequester inhibitory zinc ions from the Caspase-3 (CASP3) zymogen allowing CASP3 to auto-mature. TNF-related apoptosis-inducing ligand (TRAIL) is a pro-apoptosis ligand that can bind membrane-bound death receptors triggering the extrinsic apoptotic pathway. We hypothesise that combining PAC1 with exogenous TRAIL will significantly heighten biologic effect thereby reducing disease at doses lower than those required by either agent alone. Methods: GCT cell line KGN was treated in vitro with either a 6-log range of PAC1, or 6-log range of TRAIL concentration to establish dose-response curves. Calculated EC50 values for both PAC1 (20 μ M) and TRAIL (10 ng/mL) were then used to evaluate the biologic response to simultaneous treatment with PAC1 and TRAIL, or TRAIL delayed 24 hours after PAC1 treatment. Separately, cells from fresh patient tumour samples were cultured in vitro for 5 days, then treated in a similar fashion and assayed for viability and caspase activity. Results: Assays with PAC1 strongly reduced viability of KGN cells in a dose-sensitive manner ($p < 0.05$). Similar assays with TRAIL only reduced viability of KGN cells at the highest concentration tested (1 μ g/mL). Assays also suggest a ~24 hour delay in PAC1 reduction of GCT viability while TRAIL appears to induce a time-limited response. Using EC50 concentrations for both PAC1 (20 μ M) and TRAIL (10 ng/mL), assays with both KGN and patient-derived primary GCT cells showed that combination of PAC1 with TRAIL was dramatically more cytotoxic than TRAIL or PAC1 treatment alone ($p < 0.05$). Conclusion: Combining CASP3 activator PAC1 with apoptosis-inducing agents may be an effective strategy for treatment of GCT and warrants preclinical assessment.

DIANA DIAZ DUSSAN

Engineering

Chemical Engineering/Oncology

Nanotheranostics to manage Hypoxic Cancerous Tumors

Diana Diaz Dussan, Ravin Narain, Piyush Kumar, Kevin Peng

Hypoxic-cancerous tumors are invasive and metastatic. Genetic changes associated with hypoxia impair oxygen and drug delivery (chemotherapy) that lead to ineffective targeting, undesirable toxicity to healthy tissues and therapy failures. Clinically relevant biomedical applications of bioconjugates to deliver anticancer drugs offer a highly innovative opportunity for multimodal (imaging, chemotherapy, radiosensitization and molecular radiotherapy [MRT]) molecular theranosis (therapy+diagnosis) of solid hypoxic tumors. Developing novel stimuli-responsive carbohydrate-based nanogels – where hydrodynamic size and core composition have played key roles in enhancing drug-loading capacity and time-controlled release of anticancer drugs - provides a unique platform to address this problem. Although colloidal bioconjugates have witnessed an explosive progress in a variety of biomedical applications as drug delivery vehicles, they must still overcome some challenges to act as competitive carriers. Previous research has tried to solve issues such as efficient therapeutic loading capacity, targeted and specific delivery and controlled release profile by developing innovative glycopolymers, which produced several benefits including enhanced specificity, improved solubility and high biocompatibility. Proof-of-principle studies in our laboratory have confirmed that the encapsulated form of iodoazomycin arabinofuranoside (IAZA), an established radiopharmaceutical for imaging hypoxic tumors in cancer patients and now under exploration for cancer therapy, improved the bioavailability of the drug in hypoxic cancer cells and enhanced its radiosensitization potential. These have demonstrated high drug-loading capacity and time-controlled release. My proposed research will evaluate the targeted drug delivery efficacy of functionally modified carbohydrate-based nanogels as a theranostic approach (diagnosis and therapy) in hypoxic resistant cancer tumor cells by encapsulation of a radiolabeled clinical drug iodoazomycin arabinofuranoside (IAZA), used for treatment of these hypoxic regions.

Brennen Dobberthien
Medicine and Dentistry
Oncology - Medical Physics

Resolving the Glutamine Resonance with an Optimized Magnetic Resonance Spectroscopy
PRESS Sequence at 9.4 T

Brennen J. Dobberthien, Anthony G. Tessier, B. Gino Fallone, Atiyah Yahya

The levels of glutamate (Glu) and glutamine (Gln) are relevant to the study of cancer. Proton Magnetic Resonance Spectroscopy (MRS) can be used to quantify Glu and Gln levels. The field strength of 9.4 T offers increased spectral resolution; however, there is spectral overlap between the signals of Gln (≈ 2.45 ppm) and N-acetylaspartate (NAA; ≈ 2.49 ppm). Point RESolved Spectroscopy (PRESS) is a commonly employed technique in in-vivo MRS. By optimizing the PRESS echo times (TE1 and TE2), the J-coupling interactions of Glu, Gln, and NAA can be exploited to minimize NAA signal while retaining sufficient Glu (≈ 2.35 ppm) and Gln signal to detect both simultaneously. The peak areas of Glu, Gln, and NAA were simulated to find an optimal PRESS {TE1, TE2} combination to detect Glu and Gln. The timings were verified experimentally with a 9.4 T animal MRI scanner on phantom solutions. Two spherical phantom solutions, both containing Gln, one of which also contained Glu and NAA, were scanned to analyze the Gln signal with and without the presence of other metabolites. The optimal timing set was verified in vivo on the brains of four Sprague Dawley rats and analyzed with LCmodel software. An optimal {TE1, TE2} combination was determined to be {106 ms, 16 ms}, which resulted in simulated normalized (to the maximum) peak areas for Glu, Gln, and NAA, of 54%, 42%, and -2%, respectively. In the phantom spectra, the Gln yield at {106 ms, 16 ms} was found to be $\approx 54\%$ of the corresponding {12 ms, 9 ms} value and changed by $<10\%$ with the addition of other metabolites at {106 ms, 16 ms}. Spectra from rat brain showed individual, well resolved peaks for Glu and Gln. Reported LCModel Cramér-Rao Lower Bound (CRLB) values for all rats were $<8\%$ and $<19\%$ for Glu and Gln, respectively.

Indrani Dutta

FoMD

Experimental Oncology

Can Gamma Delta T Cells Target Breast Cancer Stem Cells?

Dutta I, Medina A, Postovit LM and Siegers GM.

Gamma Delta T cells (GDTc) comprise 2-5% of circulating lymphocytes. Immunosurveillance cells that are garnering great interest for their anti-tumoral activity, GDTc recognize antigens directly, without the need for major-histocompatibility-complex (MHC) presentation, thereby enabling rapid response. Among other antigens, GDTc respond to self-molecules signaling cellular stress; recognition and tumour lysis is mediated by the T cell antigen receptor (TCR) and/or the natural killer receptor NKG2D. NKG2D ligands include UL16-binding proteins (ULBP) 1–4 and MHC-like proteins MICA/B, which are often upregulated on transformed cells. Clinical trials have confirmed the safety of GDTc in cancer therapy, administered via adoptive transfer or in vivo stimulation with aminobisphosphonates. However, we cannot cure cancer if we fail to target cancer stem cells (CSC), the small population of cells responsible for tumour maintenance, resistance to cancer therapies and cancer recurrence. Few studies thus far have reported the ability of GDTc to target CSC (colon cancer and ovarian). We are investigating whether GDTc can target breast cancer CSC. We chose SUM 149 cells as our model, comprising an average of 10% CD24^{low}CD44^{high} CSC, allowing us to sort CSC- and non-CSC targets. GDTc cytotoxicity against adherent MCF-7 (2-D) cultures versus mammospheres, enriched for stem-like cells, was also tested. Calcein AM cytotoxicity assays show that the CSC fraction of SUM149 cells and MCF-7 mammospheres are resistant to GDTc killing compared to non-CSC SUM149 and 2-D MCF-7 cells. We have also quantified the expression of NKG2D ligands on CSC and non-CSC to determine whether their levels correlate with susceptibility to GDTc cytotoxicity. The SUM149 CSC fractions have lower expression of NKG2D ligands compared to the non-CSCs. We will validate these correlations with blocking and loss-of-function assays. Determining mechanisms of CSC immune evasion may further aid in developing ways to render CSC more susceptible to the immune system.

Mohamed Eldeeb
Medicine and Dentistry
Biochemistry

N-end rule UBR1/UBR2 E3 ligases targets Proteolytically Activated Form of PKC theta for degradation.

Mohamed Eldeeb, Mansoore Esmaili and Richard Fahlman

Cellular stresses and signalling that lead to the initiation of apoptotic pathways often result in the activation of caspases or calpains which in turn leads to the generation of proteolytically generated protein fragments with new or altered functions. Mounting number of studies reveal that the activity of these proteolytically activated protein fragments can be counteracted via their selective degradation by the N-End Rule pathway. Here we investigate the proteolytically generated fragment of the PKC theta kinase, where we report the first study on the stability of this pro-apoptotic protein fragment. We have determined that the pro-apoptotic cleaved fragment of PKC-theta is unstable in cells as its N-terminal lysine targets it for proteasomal degradation via the N-end rule pathway and this degradation is inhibited by mutating the destabilizing N-Termini, knockdown of the UBR1 and UBR2 E3 ligases. Tellingly, we demonstrate that the metabolic stabilization of the cleaved fragment of PKC-theta or inhibition of the N-end rule augments the apoptosis-inducing effect of staurosporine in Jurkat cells. Notably, we have demonstrated that the cleaved fragment of PKC theta, per se, can induce apoptotic cell death in Jurkat T-cell leukemia. Our results expand the functional scope of N-end rule pathway and support the notion that targeting N-end rule machinery may have therapeutic implications.

Osama Elshenawy

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TRANSCRIPTIONAL AND POST-TRANSLATIONAL REGULATION OF CYP1A1 BY
MONOMETHYLARSONOUS ACID IN HUMAN HEPATOMA HEPG2 CELLS

*Osama H. Elshenawy, Ghada Abdelhamid, Anatoly A. Soshilov, Michael S. Denison and
Ayman O.S. El-Kadi*

Arsenic is a human toxicant and carcinogen that has been extensively studied over decades; however, no definitive understanding for underlying mechanisms has been established. Arsenic is capable of modulating the expression of aryl hydrocarbon receptor (AhR)-regulated genes, nevertheless, whether its trivalent organic metabolites have similar effects or not need to be investigated. Therefore, in this study we examined the effects of monomethylarsonous acid (MMA(III)) as compared to its parent compound sodium arsenite (As(III)) on the expression of CYP1A1 in HepG2 cells. HepG2 cells were treated with MMA(III) (5 μ M) or its parents compound As(III) (5 μ M) in the absence and presence of the prototypical AhR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 1nM). Experiments were conducted at 6 h for gene expression; 24 h for XRE-driven luciferase activity, protein expression, and EROD activity. Our results showed that both MMA(III) and As(III) decreased CYP1A1 mRNA, protein, and catalytic activity levels; and inhibit the TCDD-mediated induction of CYP1A1 mRNA, protein, and catalytic activity levels. MMA(III) and As(III) significantly inhibited XRE-driven luciferase activity and it inhibited the TCDD-mediated induction of XRE-driven luciferase reporter gene expression. Although MMA(III) and As(III) were not shown to be AhR ligands, both compounds showed inhibition of nuclear accumulation of AhR transcription factor as evidenced by immunocytochemical analysis. MMA(III) and As(III) had no effect on CYP1A1 mRNA stability; however MMA(III), but not As(III), decreased the protein stability of CYP1A1. As(III), but not MMA(III), induced HO-1 mRNA levels. Both MMA(III) and As(III) increased ROS production. Our results demonstrate for the first time that, MMA(III) down-regulates CYP1A1 mainly through transcriptional and post-translational mechanisms.

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This modulation of CYP1A1 proves that trivalent metabolites of arsenic are highly reactive and could participate in arsenic toxicity.

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Hashem Etayash

Pharmacy and Pharmaceutical Sciences

Pharmacy

Nanomechanical Sandwich Assay for multiple Cancer Biomarkers in Breast Cancer Cell-derived Exosomes

Hashem Etayash, A. R. McGeeb, K. Kaur and Thomas Thundat

Exosomes are nanoscale vesicles with sizes in the range of 30 nm – 150 nm shed by many cell types into the bloodstream, including cancer cells. As they harbour a number of bioactive receptors, nucleic acids, and signalling proteins for cell-to-cell communications, they have become increasingly attractive diagnostic and therapeutic targets. The use of exosomes as cancer diagnostic biomarkers; however, is limited by their size, heterogeneity and the need for extensive purification and labelling. In this study we report the use of cantilever sensor arrays for simultaneous detection of multiple exosomal surface-antigens with high sensitivity and selectivity. Exosomes derived from breast cancer cells were selectively identified from a human serum by targeting over-expressed membrane-proteins CD24, CD63, and EGFR. Excellent selectivity; however, was achieved when targeting the cell-surface proteoglycan, glypican-1 (GPC1) at extraordinary limits (~ 200 exosomes ml⁻¹, ~ 0.1 pg ml⁻¹). The study showed further enhanced sensitivity of the detection by using a nanomechanical sandwich assay, where antibodies-coated gold nanoparticles were introduced into the cantilever sensor. This finding is the first to identify GPC1 as a selective biomarker for breast cancer exosomes. It also offers opportunities for the development of exosomes isolation techniques for tracking breast tumors and monitor efficiency of treatments.

Clara Fallone

Oncology

Medical Physics

Assessing Fat Unsaturation Measures Obtained with In-Vivo Magnetic Resonance

Spectroscopy Techniques

Clara Fallone, Atiyah Yahya

Levels of fat unsaturation are relevant to the study of cancer. Proton Magnetic Resonance Spectroscopy (MRS) is a non-invasive method that has been used to analyze fat composition in vivo. The ratio of the olefinic (~5.4 ppm) to methylene (~ 1.3 ppm) peak areas has been employed to estimate relative levels of fat unsaturation using PRESS and STEAM in-vivo pulse sequences at 3T. Short echo time (TE) values can be employed; however if water signal obscures the olefinic resonance, optimal TE values such as 200 ms for PRESS and 100 ms for STEAM are more suitable. In this work, we compare the ratio of olefinic to methylene peak areas acquired from five oils of varying unsaturated fatty acid content at 3T, to that obtained using more accurate high-resolution MRS at 18T. Spectra were acquired with short-TE STEAM (20 ms), short-TE PRESS (40 ms) and with the optimal echo times. The average difference in olefinic to methylene ratios between the high resolution spectra and those acquired with in vivo techniques are: 2% for STEAM 20 ms, 10% for STEAM 100 ms, 6% for PRESS 40 ms, and 250% for PRESS 200 ms. Transverse (T₂) relaxation and J-coupling effects are minimized with the shorter TE values; therefore small deviations are observed for the short-TE spectra. For STEAM 100 ms, the combined signal loss of the olefinic protons due to J-coupling and T₂ compares similarly to the methylene signal loss (primarily due to T₂). The significant difference for PRESS 200 ms occurs because of the relatively larger decay of the methylene signal due to T₂ relaxation and due to less loss of olefinic signal due to J-coupling. The work indicates that short-TE and STEAM 100 ms spectra provide more reliable estimates for absolute olefinic to methylene ratios compared to PRESS 200 ms spectra.

Scott Findlay

Medicine

Oncology

A Digital PCR-Based Method for Streamlined Screening of Genome Edited Cells

Scott D Findlay, Krista M Vincent, Jennifer R. Berman, and Lynne-Marie Postovit

The rapid adoption of precision gene editing tools such as CRISPRs and TALENs for cancer research and eventually therapeutics necessitates assays that can rapidly detect and quantitate the desired alterations. Currently, the most commonly used assay employs “mismatch nucleases” that recognize and cleave heteroduplexed DNA amplicons containing mismatched base-pairs. However, these assays are prone to false positives due to cancer-associated mutations and/or SNPs and require large amounts of starting material. Here we describe a powerful alternative in droplet digital PCR (ddPCR) mutation screening that is both highly sensitive and specific. Furthermore, ddPCR assays can distinguish single-cell derived clones with a single mutated allele from those with all alleles mutated. We use this assay to detect knockout-inducing alterations to stem cell associated genes NODAL and SFRP1 in human breast cancer and melanoma cell lines, generated using either TALENs or an “all-in-one” CRISPR/Cas plasmid modified for one-step cloning and blue/white screening. Moreover, we highlight how ddPCR can be used to assess the efficiency of different TALEN-based genome editing strategies. Collectively, this work highlights how ddPCR-based screening can be paired with CRISPR and TALEN technologies to enable sensitive, specific, and streamlined approaches to gene editing and validation.

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Moses Fung

Medicine & Dentistry

Undergraduate Medical Education

Sinonasal squamous cell carcinoma and p16 immunohistochemistry from human papilloma virus infection: a systematic review and meta-analysis

Moses Fung, Adrian Mendez, Vincent L. Biron, David W.J. Côté

Sinonasal squamous cell carcinoma (SNSCC) is rare malignancy with a poor prognosis, despite recent advances in surgical and radiation therapy. Human papillomavirus (HPV)

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infection is well established in oropharyngeal squamous cell carcinoma, but its role in SNSCC remains unclear. Immunohistochemistry of tumor suppression protein p16 (p16INK4a) is a valuable marker in oropharyngeal squamous cell carcinoma and has the potential to also serve that function in SNSCC. A comprehensive literature search was performed using searches on MEDLINE, PubMed, EMBASE, Scopus, and Web of Science to review the role of p16 immunohistochemistry on HPV detection and survival measures of HPV infection-associated SNSCC. Eleven articles met the final criteria for inclusion in this study. Data pertaining to HPV status, p16 immunohistochemistry and HPV-related survival outcomes were collected. This meta-analysis suggests that immunohistochemistry of p16INK4a is a surrogate marker for HPV infection in SNSCC and that overall survival is improved in patients with HPV-positive status. This prognostic effect needs to be confirmed by larger multi-institutional studies.

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Aravindhan Ganesan

Pharmacy and Pharmaceutical Sciences

Pharmacy

Modelling the interactions of CD28:B7-1 and CTLA-4:B7-1 complexes: Towards understanding the control of immune responses against cancers.

Aravindhan Ganesan and Khaled Barakat

Activation of T lymphocytes (or T-cells) plays a central role in anti-cancer immune responses, which controls tumor initiation and progression. CD28 and CTLA-4 are transmembrane receptors that are responsible for the regulation of the second signal that is crucial for T cell activation. CD28 and CTLA-4 share ~30% sequence identity and both receptors bind to the same set of ligands, B7-1 and B7-2, from the antigen-presenting cells. Nevertheless, their interactions tend to oppositely impact the T-cell responses. The interactions of CD28 with B7 ligands deliver a co-stimulatory signal that activates T cells, thus enhancing the T cell-mediated immune responses. Conversely, the interactions between CTLA-4 and B7 ligands trigger an inhibitory signal that down-regulates and inactivates the T cells. This eventually helps in the survival and growth of cancer cells. The reasons for such contrasting functional properties of CD28 and CTLA-4, despite their structural similarities and common binding partners, are poorly understood. Furthermore, there is very little information about how these receptors interact with the same set of ligands. In this work, we perform advanced molecular

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modelling and extensive molecular dynamics (MD) simulations to characterize these interactions, at molecular-level. Ensemble-based protein-protein docking and MD-based binding-free energy calculations are performed to build the first comprehensive model of CD-28:B7-1 complex. Subsequently, extended MD simulations of CTLA:B7-1 and CD28-B7-1 complexes are performed to unravel the similarities and differences in their interactions. This will lead to significant insights for designing novel inhibitors to specifically target CTLA-4 and unleash the immune system against cancer.

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Elizabeth Garcia

Medicine

Pediatrics

STUDY OF THE IN VITRO INTERACTION OF PARTICULATE MATTER WITH HUMAN BRAIN-DERIVED ASTROCYTES: EFFECT ON THE ARYL HYDROCARBON RECEPTOR (AHR/CYP1A1) PATHWAY

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INTRODUCTION/OBJETIVES: Air pollution has recently been classified as a human carcinogen. Epidemiological and toxicological studies have pointed to particulate matter (PM) as the pollutant that explains most of the health effects related to air pollution. PM is a mixture of compounds our interest is the possible effect of polycyclic aromatic hydrocarbons (PAH). Our objective is to study the molecular mechanisms by which different PM sizes and composition may influence tumorigenic effects. Specifically, we are interested in the Aryl hydrocarbon Receptor (AhR)/CYP1A1 pathway in astrocytes. **METHODS:** Human astrocytes in culture were exposed with different PMs: sizes (2.5-10) micrometers and sites from business (BUS) and industrial (IND) areas of well characterized air-borne particles in Mexico City. Quantitative-RT-PCR was also carried out to measure gene expression levels of AHR and CYP1A1. We assess the production of TNF-alpha by enzyme-linked immunosorbent assay (ELISA). We also determined AhR and CYP1A1 cellular localization by immunofluorescence microscopy. We carried out the correlations between the composition of the samples and our results using Pearson's analysis. **RESULTS:** After exposure to PMs, astrocytes exhibited an increased expression of AhR and CYP1A1 as compared to the control

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(unexposed cells). When using PM_{2.5} there was a significantly higher expression of AhR and CYP1A1 induced by BUS as compared to IND. However, PM₁₀ didn't exhibit a significant difference between BUS and IND. PM showed variations in pro-inflammatory effects (TNF- α) depending on the composition being soil the triggering component. We also observed the translocation of the AhR receptor to the nucleus and an increased expression of CYP1A1 with PM_{2.5} and PM₁₀. CONCLUSIONS: The level of expression of the AhR and CYP1A1 genes depended on the size and origin of PMs. These data demonstrate that PMs participate as a xenobiotic in an AhR-mediated signal transduction pathway, depending on PM composition.

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Francesco Gentile

Science

Physics

A Novel Model Of The Human XPF Nuclease Domain For The Rational Design Of DNA Repair Inhibitors

Francesco Gentile, Jack A. Tuszynski, Khaled H. Barakat

XPF-ERCC1 is a vital member of the nucleotide excision repair (NER) pathway and the inter-strand crosslink DNA repair pathway. This heterodimeric protein complex is responsible for the cleavage of the 5' terminal around bulky DNA lesions. Although its activity is essential to maintain genome integrity in healthy cells, it counteracts the induced DNA damaging actions of many chemotherapy agents such as cisplatin. The pharmacological inhibition of the XPF-ERCC1 activity is, therefore, considered as an innovative approach to overcome drug resistance in cancer cells and can be used in combination with current cancer treatments to improve their efficacy. Hence, this complex represents a promising target for structure-based drug design. The active site of the XPF endonuclease involves 155 amino acids and is characterized by a conserved V/IERKX3D metal-binding motif. There is no experimentally determined structure of this domain to date and although there are a few inhibitors that have been recently reported, the insights on their mode of binding to XPF is still lagging. In this work, we have employed several bioinformatics tools to identify sequence and structure similarity among all XPF-related structures within the protein data bank (PDB) database. An ensemble of structures of the nuclease domain has been obtained and refined using long molecular dynamics (MD)

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simulations and iterative clustering of the trajectories. A subset of known XPF-inhibitors has been also docked within the modeled active site, providing, for the first time, detailed indications about their binding modes. This model provides a detailed characterization of the catalytic site including the bound metal ions, and forms a starting point to rationally design novel and more potent XPF inhibitors.

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Nidhi Gupta

Laboratory Medicine and Pathology

Laboratory Medicine and Pathology

High Myc transcription activity characterized a small sub-set in triple negative breast cancer that carries cancer stem-like features.

Nidhi Gupta, Karen Jung, Chengsheng Wu, Abdulraheem Alshareef, Bassam S. Abdulkarim and Raymond Lai.

Background We have previously identified a novel dichotomy within triple-negative breast cancer (TNBC) cell lines and patient tumors, based on their differential responsiveness to a Sox2 regulatory region 2 (SRR2) enhancer sequence reporter. Reporter responsive (RR) cells were more stem-like and tumorigenic than reporter unresponsive (RU) cells. However, the protein drivers of the RR cells are unknown in TNBC cells. Using this model, we aimed to identify novel regulators of intra-tumoral heterogeneity in TNBC. **Methods** Using bioinformatics to analyze the protein binding consensus sequences of the SRR2, we identified Myc oncoprotein as one of the regulators of SRR2 reporter activity. Myc transcription activity and its binding to the SRR2 probe were measured using immunoprecipitation and chromatin immunoprecipitation-qPCR assays. Stem-like features were evaluated by using mammosphere assays, chemoresistance assays and flow cytometry assays measuring the CD44+/CD24- immunophenotype. The prognostic significance of Myc was evaluated in 35 cases of TNBC patient tumors. **Results** Myc protein was highly expressed and highly transcriptionally active in the RR cell subset compared to the RU cell subset within TNBC cell lines. Enforced MYC expression in RU cells resulted in a significant increase in SRR2 reporter activity, Myc-DNA binding ability, proportion of CD44+/CD24- cell population, cisplatin chemoresistance and mammosphere capability. Differentially higher ERK activation in RR cells contributed to their high Myc expression, as pharmacologic inhibition of ERK resulted in substantial decreases in

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Myc expression, SRR2 reporter activity and colony formation ability. Furthermore, Myc was differentially expressed and co-localized with CD44 in primary patient tumors. Lastly a significant correlation was observed between higher Myc expression and shorter overall survival (N=35; p<0.05). Conclusions In conclusion, differential Myc expression underlies the phenotypically-distinct breast cancer cell subsets in TNBC, and inhibition of the MAPK/ERK/Myc axis can be an effective approach in eliminating these more tumorigenic cell subsets in TNBC.

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Amr Hamour

Medicine

Surgery

High-Definition Video Modules: A Validated Novel Approach for Teaching Thyroid Cancer Surgery

Amr F. Hamour, Adrian I. Mendez, Jeffrey Harris, Vincent L. Biron, Hadi Seikaly, David W. J. Côté

Objectives: Video teaching modules have been shown to be effective tools in surgical education, complementing traditional post-graduate curricula. Unfortunately, there is a lack of validated modules described in the literature, specifically for teaching thyroidectomy. The primary objective of this study was to develop and validate a high definition video-based teaching module instructing thyroidectomy surgery to Otolaryngology – Head and Neck Surgery trainees. Methods: This prospective study included 7 intermediate to senior Otolaryngology – Head and Neck Surgery residents. Each consented participant first performed a hemi-thyroidectomy, serving as the initial assessment. After a washout period of at least 2 weeks, each participant was given the teaching module. The 15-minute module was developed using a three-camera system and detailed a step-by-step approach to the surgery. After exposure to the module, each trainee then performed the same procedure. Recordings of both procedures were de-identified and reviewed by an independent evaluator. Scoring was done using the Observational Clinical Human Reliability Assessment (OCHRA) system. Results/Discussion: A statistically significant decrease in error occurrence was found after exposure to the teaching module. In addition, the number of staff takeover events was less in the post-exposure group as compared to the pre-exposure group. This difference was found to

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be statistically significant. Conclusion: High-definition video teaching modules are a useful complement to traditional surgical training. Otolaryngology – Head and Neck Surgery resident trainees experienced a significant reduction in both errors committed and staff takeover events when performing a thyroidectomy after exposure to the teaching module.

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Zahra Havalishahriari

Medicine

Biochemistry

Studying substrate specificity for the DNA repair enzyme, poly-nucleotide kinase/phosphatase (PNKP)

Zahra Havalishahriari and Mark J. N. Glover

Polynucleotide kinase/phosphatase is an enzyme involved in two major DNA repair pathways, single strand break repair and non-homologous end joining. Two catalytic domains of this enzyme, kinase and phosphatase, take a role in converting the non-ligatable DNA lesion to re-joinable strands at the break sites. It also interacts with other proteins in DNA repair pathways. PNKP defect has been linked to neurological disorders and cancer. Hence, small molecule inhibitors that specifically inhibit the enzyme activity will have a potential role in cancer treatment. The phosphatase domain has been suggested to be the most important target, as 3'-phosphate is one of the major lesions at the IR-induced strand breaks. Understanding substrate specificity of the phosphatase domain will help us to rationally design the high throughput screening (HTS) assay of the enzyme with small molecule libraries and finally to construct an inhibitor that selectively impede PNKP catalytic activity. In order to achieve these goals we studied substrate specificity of the PNKP phosphatase domain with fluorescent polarization and electrophoretic mobility shift assay (EMSA). By using different DNA substrates, in the forms of single or double stranded molecule and in a variety of lengths, we found that the enzyme has a tighter binding affinity for double stranded DNA substrates and loses its specificity towards 3'-phosphate end by increasing the length of the single stranded substrates. We also tested PNKP mutants to determine which regions of the enzyme are important for strand recognition and energy compensation upon unwinding of double stranded DNA substrates. These data have shed light on drug screening and design for PNKP.

Ruicen (David) He
Medicine and Dentistry
Oncology

Aging in Human Cells is Linked to BubR1 Expression

Ruicen He, Cody W Lewis, Gordon KT Chan

BubR1 is a mitotic kinase well-studied in preventing premature anaphase before all chromosomes have attached to spindles and are aligned in the middle of the dividing cell. In 2004, Jan Van Deursen published a study that revealed two things: BubR1 levels decreased in mice as they aged; and mice that were hypomorphic for BubR1 aged prematurely. In following years, Van Deursen showed that increasing BubR1 levels in mice prolonged their lifespan. However, these studies have never been tested on human cell lines; and it is still unknown if is a correlation between decreasing levels of BubR1 and aging in human cells exists. In this study, four non-immortalized human fibroblast cell lines were examined for BubR1 expression over time. The WI-38 and HGFDFN168(FN168) were used as normal aging models; and the HGADFN167(FN167) and HGADFN169(FN169), two progeroid cell lines, were used as accelerated aging models to compare to the normal cells. An immortalized cell line, HeLa, was used as a control for its known BubR1 expression- which remains constant through time. Cells were passaged over time and cell extracts were made after each split. The cell extracts were analyzed for BubR1 expression by western blot. Preliminary results show that BubR1 levels declined in all non-immortalized cells as they were passaged. In addition, the accelerated aging models exhibited lower BubR1 levels when compared to age-matched normal cell models. These findings support Van Deursen's work in mice; that BubR1 expression is correlated with aging.

Maria Sol Herrera-Cruz

Medicine and Dentistry

Cell Biology

Investigating Rab32's role as a negative prognostic marker for breast cancer

Maria Sol Herrera-Cruz, Thomas Simmen

The mitochondria-associated membrane (MAM) is a subdomain of the Endoplasmic Reticulum (ER) in close apposition with mitochondria. In recent years, the MAM has emerged as an important signalling hub for processes regulating cell fate, and, unsurprisingly, has been linked to cancer. Our lab has identified the GTPase Rab32 as a MAM-enriched protein involved in several processes, including calcium signalling, apoptosis onset, mitochondrial dynamics, and autophagy. Through collaboration, our lab has demonstrated that high Rab32 mRNA levels correlate with poor survival probability and high tumor mitotic grade in breast cancer patients. Furthermore, Rab32 is upregulated in almost 25% of breast cancers, making it a relevant potential biomarker for this disease. Our findings suggest Rab32 relocalizes the pro-apoptotic Bcl-2 family protein Bim to the MAM, where Bim becomes targeted for autophagic degradation. Decreased Bim levels are known to support tumor growth and metastases. Hence, at least in part, Rab32 appears to be mediating its negative effect in breast cancer patients through this autophagic degradation of Bim. In brief, our work has provided insights into Rab32's role in regulating cell fate processes occurring at the MAM in order to understand its effects in breast cancer and to lay a foundation for its potential use as a biomarker for this disease.

Cameron Hough

Medicine and Dentistry

Oncology - Medical Physics

Investigating Non-ionizing Terahertz Radiation for Novel Cancer Therapies via Genomic Pathway Analysis

Cameron Hough

Terahertz (THz) radiation occupies a broad band of the electromagnetic (EM) spectrum between microwave and infrared energies, and is therefore non-ionizing. The frequencies involved ($\sim 10^{12}$ Hz) couple strongly to natural oscillations of hydrogen bonds, which are responsible for the conformation of essential biological structures such as nucleic acids and proteins. The high sensitivity of THz radiation to molecular changes allows for excellent contrast between diseased and healthy tissue in diagnostic spectroscopy and imaging, and this has been utilized in the early detection of skin cancer and as an intra-operative tool during breast cancer surgery. However, recent studies have shown that interactions with hydrogen bond networks can create/amplify bubbles in the DNA strand and alter the conformational states of proteins, which has been shown to influence genetic expression and affect protein structure/function. These observations imply the potential for: (1) significant health risks, requiring the establishment of safe exposure limits; and (2) a novel therapeutic modality, for which a characterization of the tissue response is required to predict disease control. Intense THz pulses are single-cycle EM oscillations with very high peak electric fields. These pulses are focused onto 3D human skin tissue models and the resulting gene expression is analyzed at the transcript and protein levels. The list of significantly differentially expressed genes is used to identify deregulated molecular signaling pathways. The preliminary data suggests that exposure to THz radiation could significantly deregulate signaling pathways commonly mutated/over-active in many human cancers. This activity shows that THz radiation could have either excitatory or inhibitory roles in cellular proliferation, indicating the potential for both tumor-suppressing or oncogenic effects. Studying tissue responses for varying exposure parameters will allow us to determine thresholds at which biological consequences become significant, assist in establishing safe exposure levels, and elucidate the potential for a novel therapeutic modality for cancer.

Yung-Hsing Huang
Medicine and Dentistry
Laboratory Medicine and Pathology

Myeloma cells exhibit higher dependence and activity of STAT3 signaling in reconstructed bone matrix

Yung-Hsing (Winston) Huang and Raymond Lai

Multiple myeloma (MM) is the second most common hematologic malignancies in North America yet remain incurable in most cases. As a cell type originated from bone marrow matrix, myeloma cells are known to be interacting with a variety of cell types and extracellular matrix (ECM). These interactions contribute significantly to the pathogenesis and chemoresistance in MM. Conventional culture (2D) cannot resemble such conditions for MM cells to behave as in real bone marrow. Here, a previously established reconstructed bone marrow (rBM) three-dimensional (3D) culture was adapted as a model for MM molecular biology study. Comparing 5 different signaling pathways that are commonly complicated in MM cells (STAT3, ERK, Akt, NF κ B and Notch) cultured in 2D versus 3D by western blot analysis, it was routinely found that the STAT3 signaling was activated in 3D MM cells. The sustainability and viability of 3D MM cells in rBM 3D culture is highly dependent on the initial cell number. We found that 100,000 MM cells resulted in viable cells with sustainable STAT3 activity 48 hours after seeding in 3D culture up to 4 days. Treatment of STAT3 inhibitor such as Stattic and S3I-1757 in 3D MM cells resulted in more cytotoxicity in 2D MM cells. Moreover, treatment of bortezomib (a common MM therapeutic) in combination with Stattic results in lower IC₅₀. These results suggest that MM cells are more dependent on STAT3 activity in bone marrow mimicry. Moreover, targeting STAT3 can be a promising strategy for improved MM therapy.

Sherif Idris

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Department of Surgery

Investigation of EZH2 Pathways for Novel Epigenetic Treatment Strategies in Oropharyngeal Cancer

Sherif Idris, MD; Cameron Lindsay, BSc; Morris Kostiuik, PhD; Colin Andrews, BSc; David W.J. Côté, MD, MPH, CCFP, FRCSC; Daniel A O'Connell, MD, MSc, FRCSC; Jeffrey Harris, MD, MHA, FRCSC; Hadi Seikaly, MD, MAL, FRCSC; Vincent L. Biron, MD, PhD, FRCSC

Background: In recent decades, the incidence of oropharyngeal squamous cell carcinoma (OPSCC) has been rising worldwide as a result of increasing oncogenic human papillomavirus (HPV) infections in the oropharynx. EZH2 is an epigenetic regulatory protein associated with tumor aggressiveness and negative survival outcomes in several human cancers. We aimed to determine the role of EZH2 as a potential therapeutic epigenetic target in HPV-positive and negative OPSCC. **Methods:** The expression of EZH2 was measured by immunohistochemistry (IHC) and droplet digital PCR (ddPCR) in 2 HPV-positive and 2 HPV-negative cell lines. The cell lines were then cultured and treated with one of 3 EZH2 epigenetic inhibitors (3-deazaneplanocin A, GSK-343 and EPZ005687) or DMSO (control). Following 7 days of treatment, cells were analyzed and compared by gene expression, cell survival and proliferation assays. **Results:** EZH2 targeting resulted in greater inhibition of growth and survival in HPV-positive compared to HPV-negative cells lines. The expression profile of genes important in OPSCC also differed according to HPV-positivity for Ki67, CCND1, MET and PTEN/PIK3CA, but remained unchanged for EGFR, CDKN2A and p53. **Conclusion:** Inhibition of EZH2 has anti-tumorigenic effects on OPSCC cells in culture that is more pronounced in HPV-positive cell lines. EZH2 is a promising epigenetic target for the treatment of OPSCC.

Andre Isaac

Medicine and Dentistry

Surgery

Droplet Digital Polymerase Chain Reaction for Detection of Oncogenic Human Papillomavirus in Oropharyngeal Swabs: x000B A Prospective Cohort Study

Andre Isaac, Morris Kostiuk, Han Zhang, Cameron Lindsay, Fawaz Makki, Jeffrey R Harris, Daniel A O'Connell, David WJ Cote, Hadi Seikaly, Vincent L Biron

Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) caused by oncogenic human papilloma virus (HPV) is rising worldwide. HPV-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 minimally invasive assay for detection of HPV-16 based on oropharyngeal swabs using ddPCR. Methods: This was a prospective cohort study of patients with biopsy-confirmed p16 positive OPSCC at a tertiary care Otolaryngology-Head and Neck Surgery referral center. Two groups of controls were used: patients with p16 negative OPSCC and patients who underwent tonsillectomy for a benign indication. Each patient underwent an oropharyngeal swab followed by RNA extraction 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, as well as RT-qPCR. The sensitivity and specificity of the novel assay was calculated against p16 IHC. Results: 120 patients were included, of which 40 were healthy controls. P16 positivity was present in 85 % of oropharyngeal tumors. The specificity and sensitivity of ddPCR for oropharyngeal swabs was 100 % and 92 % respectively, using 20-50 times less RNA than that required for conventional RT-qPCR. Conclusion: ddPCR of HPV-16 E6/E7 using oropharyngeal swabs is a rapid and ultra-sensitive method of detecting HPV-16 in OPSCC without requiring a tissue biopsy.

Saket Jain

Faculty of Medicine and Dentistry

Oncology

Role of Transcription factor AP-2 in Glioblastoma Pathogenesis

Saket Jain, Rongzong Liu and Roseline Godbout

Glioblastoma (GBM) are highly aggressive brain tumours. Patients diagnosed with GBM have a dismal prognosis, with a median survival of 15 months. Activating Protein 2 (AP-2) is a family of transcription factors (AP-2a, b, c, d and e) involved in the regulation of genes responsible for early development, cellular growth and differentiation. The subcellular localization of AP-2a has been associated with astrocytoma tumor grade. In low grade astrocytoma, AP-2a is primarily found in the nucleus, whereas in GBM, it has a cytoplasmic pattern. Furthermore, our immunofluorescence and nuclear-cytoplasmic fractionation analyses indicate that AP-2b localize to the cytoplasm of GBM cells. We are particularly interested in AP-2b as its expression correlates with poor survival in GBM patients. We are using patient-derived tumor neurospheres as our experimental model. Our data reveals that AP-2b is more highly expressed in neurosphere cultures as compared to adherent cultures derived from the same patients. Interestingly, AP-2b localizes to the nucleus of neurosphere cultures.

Knockdown of AP-2b in GBM neurosphere cultures results in decreased expression of stem cell markers SOX2, as well as decreased cell migration. Stem cell maintenance and mesenchymal characteristics are associated with hypoxia in GBM. We observed an increase in AP-2b levels in GBM neurospheres cultured in 0.5% O₂. Our preliminary immunohistochemical analysis of GBM tumor tissue sections show expression of AP-2b in pseudopalisading cells surrounding necrotic regions. These combined data suggest that AP-2b may regulate stem cell maintenance and migration in GBM. Our future objectives are to determine: (i) the mechanisms by which AP-2 affects the growth properties of GBM cells, and (ii) the mechanism by which AP-2 subcellular localization is regulated and how it affects stem cell maintenance in GBM. We will also examine how AP-2 expression affects GBM response to radiation and chemotherapeutic agents such as temozolomide.

David Jenish
Medicine and Dentistry
Oncology

The Alberta Pre-Phase I Cancer Program

David Jenish, Dr. JR Mackey

The Alberta Pre-Phase I Cancer Program provides a framework for translating promising therapeutics from discovery to their evaluation in a Phase I clinical trial. We answer the question of “What comes next?” for the discovery scientist, helping them navigate the logistics and the regulatory path to first-in-human clinical testing of their promising cancer therapeutic. The Program is administered and guided by a Steering Committee which includes expertise in drug discovery, drug development and clinical oncology, and includes representation from the Alberta innovation community. While centred at the University of Alberta, the Program is provincial in scope. The Program’s focus is primarily oncology therapeutics arising within the universities. Projects outside of oncology and outside of the universities will be considered. The Program is designed to complement and leverage existing services by being part of the innovation community network, both within and outside of the university. The translation of discovery science to the clinic requires careful attention to, and balancing of, often-competing scientific, regulatory and commercial concerns. Our aim is to quickly enable investigators in their work by providing guidance along this path, and by directing them to appropriate resources and service providers. Our location at the Cross Cancer Institute allows for ready access to the expertise, support and facilities required for preparing for and conducting a Phase I clinical trial. By maintaining contacts within the pharmaceutical industry, the Program can also help transition projects to industry at the earliest or most appropriate time. The Alberta Pre-Phase I Cancer Program is funded by the Alberta Cancer Foundation.

Michael Jewer

Oncology

Experimental Oncology

The role of Microenvironment Stresses and Pharmacological Agents in promoting the acquisition of the plastic phenotypes required for metastasis and chemo-resistance.

Jewer Michael, Zhang Guihua, Liu Jaihui, Vincent Krista, Taylor Meghan, Hughes Chris, Postovit Lynne-Marie

Microenvironment stresses and the pharmacological agents that mimic these stresses promote the acquisition of the plastic phenotypes required for metastasis and chemo-resistance. In order to grow and metastasise a cells must adapt to stresses like hypoxia and chemotherapy. This adaptability is mediated by cancer cells co-opting normal stem cell pathways by reactivating core pluripotency genes that lay dormant in most adult tissues. One such gene is Nodal. Nodal is a secreted protein that directs patterning during embryogenesis and regulates bivalent histone marks, promotes metastasis and poor clinical prognosis. Our current work demonstrates that stress pathways contribute to the acquisition of plasticity and aid in the development of putative Breast Cancer Stem Cell. Under stress that causes energy deficits, like hypoxia, global levels of mRNA translation are reduced to restore homeostatic energy balance. Concurrently, selective translation of mRNA result in an adaptive pro-survival protein response. We hypothesize that the acquisition of stem cell-like plasticity is a hallmark of this adaptive response. We further theorize that Nodal, which has been previously demonstrated to increase expression in response to hypoxia, promotes plasticity as part of the adaptive stress response. Two major pathways control global rates of translation, the mTOR and PERK/eIF2alpha pathway. In hypoxia both pathways act in concert to decrease global rates of translation and mount the pro-survival protein response. Here we examine post-transcriptional mechanisms by which hypoxia regulates Nodal and plasticity by: measuring the activity of the pathways regulating translation, EMT, and plasticity; assessing protein stability; and evaluating ribosome binding to mRNA termed polysome profiling. Nodal's stability increased in hypoxia. The mTOR and eIF2 α pathways are active in hypoxia and regulate stem-cell-like properties. These changes increase EMT and pluripotency gene sets leading to an

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increase multiple cancer phenotypes through the support and maintenance of cancer stem cells and contribute to metastasis.

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Bingcheng Jiang

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Study of Polynucleotide Kinase/Phosphatase (PNKP) Mutations Found in a Patient with Microcephaly, Seizures, and Developmental Delay (MCSZ) and Glioblastoma

Bingcheng Jiang, Chibawanye I. Ene, Bonnie Cole, Jeff Ojemann, Sarah Leary, Mesfin Fanta, Sudip Subedi, Michael Weinfeld

The enzyme polynucleotide kinase/phosphatase (PNKP) plays a key role in DNA repair by resolving the chemistry at DNA strand breaks. Mutations in PNKP (chromosome 19q13.4) are known to cause MCSZ, a serious neurodevelopmental disorder, but to date there has been no link to cancer initiation or progression. However, a child with MCSZ recently presented at Seattle Children's Hospital with a 3-cm glioblastoma. The child was shown to have two germline mutations in PNKP. To study the effects of the PNKP mutations found in this patient, we generated mutant PNKP cDNAs carrying either the individual mutations or the double mutation using site directed mutagenesis. These cDNAs were incorporated into bacterial and mammalian expression vectors. The bacterially expressed mutant proteins as well as the wild type have been purified and are undergoing testing for PNKP DNA kinase and phosphatase activity. The PNKP cDNAs, fused to GFP, were expressed in HeLa and HCT116 human cancer cell lines. High-content analysis and micro-irradiation techniques are being used to determine PNKP localization within the cells and recruitment to damaged DNA. Our preliminary results indicate that the mutations alter the ratio of nuclear to cytoplasmic PNKP compared to the wild-type protein.

Gurnit Kaur

Medicine and Dentistry

Medical Sciences (Laboratory Medicine and Pathology)

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

Gurnit Kaur, and Elaine M. Leslie.

Arsenic and selenium are toxic compounds, however in vivo exposures to arsenite and selenite result in mutual detoxification. The molecular basis of 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; >150 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. 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 ABCC2 SNPs that display reduced transport activity and mislocalization may not benefit from selenium supplementation.

Nadia Kobagi
Medicine & Dentistry
School of Dentistry

Are Canadian Registered Dental Hygienist Conducting Oral Cancer Screening?

Nadia Kobagi, Alix Clarke, & Minn Yoon

Purpose: Dental hygienists can be instrumental in detection of suspicious oral lesions. Early diagnosis of oral cancer is critical to increasing mortality rates. This study investigates whether Canadian dental hygienists are routinely conducting oral cancer screenings as part of their process of care. The ability to discuss sensitive topics with patients and factors that enable to impede the provision of Oral Cancer Screenings (OCSs), are also examined. Methods: A web-based survey was created, pre-tested, and validated before dissemination. An email containing a link was sent out nationally through numerous provincial dental hygiene colleges and associations. Results: 256 completed surveys were used for analysis, primarily from Ontario (n = 135), Alberta (n = 44) and Nova Scotia (n = 42). Dental hygienists conducted OCSs as part of their process of care 64%. The average reported time to complete an OCS was 4 minutes, with the majority agreeing this was sufficient time to conduct the screening (57%). Intraoral components in an OCS were examined 96% of the time in comparison to extraoral components at 73%. Greater percentage of dental hygienists with a bachelor degree reported feeling prepared by their education than those with a diploma (p = .002). Only 37% felt their education prepared them to discuss sensitive topics with their patients. Forty-three percent of respondents felt confident in their HPV knowledge and comfortable discussing HPV risk factors with patients. Conclusion: Canadian dental hygienists are regularly conducting OCSs. However, they lack comfort discussing sensitive topics such as transmission of HPV.

David Kramer
Medicine & Dentistry
Biochemistry

Characterizing the Proteomic Profiles of Human Estrogen Receptor-Positive Breast Cancers for the Development of Novel Prognostic Biomarkers

David Kramer, Yifei Wu, Judith Hugh, Richard Fahlman

In Canadian women, breast cancers account for 26% of new cancer diagnoses and 14% of cancer-related deaths each year. While breast cancer survivorship has increased over the past several decades, there is still a need for better indicators of prognosis at the time of diagnosis. ER+ breast cancers, the most common subtype, account for ~75% of all breast cancers, and can be further subtyped as Luminal A or B. Luminal A cancers have the best prognosis; these tumours typically present later in life with well-defined and localized tumours that tend to respond well to anti-hormone therapy, resulting in high rates of survival with low rates of disease recurrence. Conversely, Luminal B patients present at a young age, often with large, poorly defined tumours and are node-positive; these tumours tend to be more resistant to anti-hormonal therapies, may present with concurrent HER2/neu expression, and treatment often requires systemic adjuvant chemotherapy. Currently, distinguishing Luminal A from HER2-negative Luminal B in the clinical setting is challenging and often inconclusive. As the first-line treatment for all ER+ breast cancer patients is tamoxifen, Luminal B patients tend to have higher rates of recurrence and lower rates of survival compared to Luminal A patients. With the advent of mass spectrometry-based proteomics, it is possible to identify and quantitate thousands of proteins from a single tissue sample. Characterization of the proteomic profiles of patient-derived ER+ breast tumours, whose long-term outcomes are known, may identify biomarkers that definitively predict patients' disease prognosis and therefore the best treatment plans. Here we describe the quantitative proteomic analyses of 19 pathologically matched luminal A and B ER+ tumours. Using this data in conjunction with the patients' clinical outcomes at 80 months, we outline several candidate proteins which may potentially be used to easily distinguish between luminal subtypes of ER+ breast cancers.

Laura Lee

Medicine and Dentistry

Oncology

Hypoxia and Nodal: Epigenetic Modulators in the Development of Cancer Cell Plasticity

Lee LJ, Postovit LM.

Nodal, an embryonic morphogen, and hypoxia contribute to pluripotency in Embryonic Stem Cells (ESCs), but also support cancer progression when its 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 was used, namely breast cancer cell lines, T47D and MDA-MB-231. To determine epigenetic changes, Chromatin Immuno-Precipitation (ChIP) with high throughput sequencing (ChIP-seq) was 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. Additionally, RNA sequencing was performed to match gene expression changes and PCR will be incorporated to validate ChIP-seq and RNA-seq results. Future work will further incorporate H9 hESC for comparison of the cancer epigenome with the primed epigenetic state present in ESCs. 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.

Emma Lee

Science

Biological Sciences

Indicators & Predictors of Urethral Strictures in Prostate Brachytherapy

Lee, Emma; Jamaluddin, Muhammad; Singhal, Sandeep; Sloboda, Ron; Usmani, Nawaid

Background & Purpose: Prostate brachytherapy is an increasingly popular treatment option for men with localized prostate cancer due to its efficacy, convenience and side effect profile. This retrospective case-control study explored the relationship between dose to the different regions of the urethra and development of brachytherapy (BXT)-related urethral stricture. **Materials & Methods:** Patients developing urethral strictures after BXT at the Cross Cancer Institute, Edmonton were identified from 2008-2014. Each case was matched with two controls that had not developed a urethral stricture according to similar International Prostate Symptom Score (IPSS), planned prostate volume, post-implant prostate V150, and post-implant prostate D90 dosimetry parameters. Stricture development was compared with clinical (i.e. age, smoking status, diabetes, hypertension, vascular disease, IPSS, hormones) and dosimetric (i.e. prostate, urethra, urethra segments (i.e. base, mid-gland, apex, extra-prostatic) variables. **Results:** 34 cases were matched with 68 controls. Higher dose was identified in the apex and apical urethra (D90 of 178 Gy (SD = 22) vs. 126 Gy (SD=17)). There was no statistical association between the two groups in terms of most clinical and dosimetric variables. There was evidence that vascular disease is associated with the formation of strictures ($p=0.001$). There was a trend approaching statistical significance between dose delivered to the peri-apical and apical urethra and the formation of urethral strictures (D30 urethra at apex of 213 Gy vs. 205 Gy, p value = 0.10). **Conclusion:** Higher doses to the urethra at the apex were identified compared to other regions of the prostate. Trends towards statistical significance were identified with patients receiving very high doses to the urethra at the apex, although none of the studied parameters were statistically significant.

Luana Leitao

Faculty of Medicine and Dentistry

Biochemistry

Selective N-end Rule Pathway inhibition through UBR-box domain

Luana Leitao, Dr. Richard Fahlman

The Ubiquitin Proteasome dependent N-end rule pathway has pivotal roles in the pathogenesis of several illnesses such as malignancies, cardiovascular disorders, and neurodegeneration. It targets N-terminal destabilizing amino acid residue-containing protein substrates for degradation. Nevertheless, recent studies demonstrated that this pathway participates in a wide range of physiological and developmental processes including spermatogenesis, autophagy, oxygen sensing, and chromosomal stability. In mammals, the most common path is the Arg/N-end rule, which the recognition of the destabilizing N-termini is mediated by the UBR proteins (UBR1/UBR2) followed by the polyubiquitination of the amino acid residues of target protein substrates, and subsequently protein degradation through the 26S proteasome. Both UBR1/UBR2 E3 ubiquitin ligases have two key recognition domains, one is known as the UBR box domain, that independently functions as binding sites for type I (basic amino acids), and the other is the N-domain type II. Recent reports revealed that some pro-apoptotic substrates have type-I basic destabilizing N-terminal residues after they are cleaved by proteases, as caspases. Correspondingly, the UBR1/UBR2 ligases reduce the pro-apoptotic signaling cascade and promote resistance of cells to apoptosis-inducing chemicals. The development of a selective inhibitor of the UBR box domain of UBR1/UBR2 could inhibit degradation of several pro-apoptotic proteins, and as a result, it would considerably sensitize cells to chemotherapeutics. In this study, we are analyzing a new chemical compound that has demonstrated protein stability for arginine n-termini kinase. Furthermore, to determine the IC50 and measure the inhibitor effectiveness, we propose a viability assay employing microscopy fluorescence that has demonstrated consistent results in cell viability after PAC1 treatment. Thus, if we can selective target the N-end rule components in an appropriate concentration, we will have more accurate therapeutic windows with higher potential and reduced side effects.

Cody Lewis

Medicine and Dentistry

Oncology

Knockdown of Myt1 sensitizes breast cancer cells to Wee1 inhibition with MK-1775

CW Lewis, GKT Chan

Mitosis is dependent on Cdk1/cyclin B activity. To prevent premature mitosis Cdk1/cyclin B is regulated by Wee1 and Myt1 kinases, which add inhibitory phosphates to Cdk1 on Tyr 15 and Thr 14 respectively. In *Drosophila*, Wee1 and Myt1 are functionally redundant and the loss of one kinase is able to compensate for the other in terms of Cdk1 regulation [1]. There are several small molecule inhibitors of Wee1 including MK-1775, which is currently undergoing phase I/II trials, but there are no small molecule inhibitors of Myt1. Knocking down Wee1 (but not Myt1) with siRNA or inhibiting its activity with MK-1775 both induces premature mitosis and prolongs mitotic exit leading to cell death in HeLa cells. These findings suggest that human Myt1 and Wee1 do not share the functional redundancy observed in *Drosophila*, at least in terms of Cdk1 regulation. As a result of studies in HeLa cells, Myt1 is often dismissed as having an essential role in regulating Cdk1 activity [2, 3]. A recent study of glioblastoma stem like cells identified Myt1 as an essential regulator of both mitosis and cell survival. We are examining if Myt1 also plays a role in regulating mitosis and cell survival in breast cancer cells. We find that breast cancer cell lines (MCF-7, SK-BR-3, T-47D, and BT-474) but not HeLa cells become highly sensitive to the Wee1 inhibitor MK-1775 when Myt1 is knockdown. Sensitivity to MK-1775/Myt1 knockdown compared to MK-1775 alone as measured by a decrease in cell viability with IC50 ranged from a 14-fold decrease in MCF-7 cells to a 2-fold decrease in SK-BR-3 and a 1.5-fold reduction in HeLa cells. We will now attempt to examine the functional role of Myt1 in the regulation of mitosis and determine if pathways that inhibit or activate Myt1 can be used to enhance MK-1775 efficacy. 1. Jin, Z., et al., *Drosophila myt1* is the major cdk1 inhibitory kinase for wing imaginal disc development. *Genetics*, 2008. 180(4): p. 2123-33. 2. Coulonval, K., H. Kooken, and P.P. Roger, Coupling of T161 and T14 phosphorylations protects cyclin B-CDK1 from premature activation. *Mol Biol Cell*, 2011. 22(21): p. 3971-85. 3. Nakajima, H., et al., Myt1 protein

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kinase is essential for Golgi and ER assembly during mitotic exit. *J Cell Biol*, 2008. 181(1): p. 89-103.

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Lei Li

Medicine and Dentistry

Oncology

DEAD Box 1 Facilitates Homologous Recombination at DNA Double Strand Breaks

Lei Li, Devon Germain, Michael Hendzel and Roseline Godbout

Evidence suggesting that RNA and RNA binding proteins are involved in cellular response to DNA double strand breaks (DSBs) is accumulating; however, we still know very little about their roles at sites of DSBs and in the DSB repair process. We found that DEAD box 1 (DDX1), an RNA unwinding protein that is overexpressed in a subset of childhood tumors and breast cancer, promotes cell survival and DSB repair post- ionizing radiation. Depletion of DDX1 results in reduced DSB repair by homologous recombination (HR). While DDX1 is not essential for end resection, a key step in homology-directed DSB repair, DDX1 is required for maintenance of the single-strand DNA once generated by end resection. Moreover, we provide direct evidence for the presence of RNA-DNA hybrids at DSBs and suggest that binding of RNA to DNA at DSBs may impact repair efficiency and repair pathway choice. We show that transcript deregulation at sites of DSBs has a significant effect on DSB repair by HR in DDX1-depleted cells and that RNA-DNA duplexes are elevated at DSBs in DDX1-depleted cells. Based on our combined data, we propose a role for DDX1 in resolving RNA-DNA structures that accumulate at DSBs located at sites of active transcription. Our findings point to a previously uncharacterized requirement for clearing RNA at DSBs for efficient repair by HR.

Cameron Lindsay
Medicine and Dentistry
Surgery

Inhibition of EZH2 in human papillomavirus positive and negative cell lines

Cameron Lindsay, Morris Kostiuk, Jeffrey Harris, Daniel A. O'Connell, Hadi Seikaly and Vincent L. Biron

Head and neck squamous cell carcinoma is the sixth leading cancer worldwide, with incidence rates of oropharyngeal squamous cell carcinomas (OPSCC) rapidly increasing due to human papillomavirus (HPV)-related infections. Enhancer of zeste homolog 2 (EZH2) is an epigenetic modifier responsible for the trimethylation of lysine 27 at histone 3 (H3K27me3) which results in gene silencing due to chromatin compaction. EZH2 is frequently overexpressed in HPV-positive cancers and has been associated with increased tumor aggressiveness, making EZH2 pathways an attractive chemotherapeutic target. To determine and compare the efficacy of EZH2 inhibition, two HPV-positive (SCC-47 and SCC-104) and two HPV-negative (SCC-1 and SCC-9) cell lines were cultured and treated with an EZH2 pathway inhibitor; GSK-343, DZNep, or EPZ-5687, at varying concentrations for 7 days. Following treatment, cells were processed for Western blot analysis to detect changes in H3K27me3 in response to epigenetic inhibitors. Inhibitors displayed variable effects depending on cell line. GSK-343 treatment resulted in H3K27me3 loss in all cell lines, however, only HPV-negative cell lines displayed demethylation with DZNep, and only SCC-1 displayed demethylation with EPZ-5687. Droplet digital PCR analysis was used to detect changes in RNA expression levels of cell proliferation markers (EGFR, MKI67, CCND1, MET, EZH2, and PIK3CA), tumor suppressors (TP53, CDKN2A, and PTEN), and cancer stem cell markers (ALDH1A1 and CD44) following treatment. Variable gene expression patterns were observed in individual cell lines following treatment with inhibitors, but trends were observed based on HPV status. In response to DZNep treatment, HPV-negative cell lines overexpressed proliferation markers, whereas HPV-positive cell lines showed decreased expression of EGFR and CD-44. Epigenetic inhibitors targeting the EZH2 pathway may provide novel chemotherapeutic strategies for oropharyngeal carcinomas. Further investigation utilizing primary tumor explant models is suggested prior to clinical assessment.

John Mackey

Medicine and Dentistry

Oncology

Geographic variation in adverse event reporting patterns in breast cancer clinical trials

Rodrigo Fresco, Valeria González, Andrés Machado, Gonzalo Spera, Pablo Millán Carlos Meyer, Helena Fung, John R. Mackey

Introduction: Adverse event (AE) reporting in clinical trials (CT) informs the safety of investigational products. Once approved, safety information in the monograph/prescribing information mainly derive from CT safety data. Most phase 3 CT in oncology are multinational. While post-marketing reporting of AE is known to vary according to geographic region, the geographic variation in AE reporting in cancer CT is unknown. Objective: To compare between several geographic regions, the number of AE and serious AE (SAE) reported in breast cancer CT conducted by TRIO. Methodology: We retrospectively reviewed AE data from two completed phase 3, multinational CT of anticancer therapies in advanced breast cancer. Participating countries were grouped in 7 regions according to their geographic location (East Asia, Eastern Europe, Latin America and Caribbean, Middle East and Africa, Non-Eastern Europe, North America, Oceania). Regions were masked and numbered from 1 thru 7. For each region we calculated the mean number of AE and SAE terms per patient and the mean number of AE and SAE per cycle/per patient. Comparisons between regions were done using unequal variance t-test (PROC TTEST, SAS 9.3). Results: 1,863 patients from 35 countries and 310 sites were included. Regions varied markedly in numbers of AE and SAEs reported. Two regions (1 and 6) reported the highest number of AEs while region 4 the lowest rates, approximately 3-fold lower than regions 1 and 6 (mean AE terms 22.75 [region 1] vs. 7.88 [region 4]; $p < .0001$). Region 6 reported approximately 9-fold higher rates of SAEs than region 4 (mean: 1.33 vs. 0.15, $p < .0001$). Conclusion: AE and SAE reporting patterns vary markedly by geographic region and one region appears to systematically under report both AEs and SAEs. These data warrant confirmation, and if confirmed, may provide an important caveat on the interpretation of reported study safety data.

Sheena MacLeod

Medicine and Dentistry

Oncology

Induction and Maintenance Regimen with Peptide Receptor Radionuclide Therapy (PRRT)

Lu-177-DOTA-TATE (Lu-177) in Patients with Advanced Neuroendocrine Tumours (NET).

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Introduction/background: PRRT using Lu-177 is a treatment option for advanced NETs. Aims: We hypothesize long-term and ongoing therapy with Lu-177 improves outcomes, is effective and safe for these patients. We evaluated toxicities of a Lu-177 regimen of induction (4 cycles of 5.6 GBq every 10 weeks) and maintenance (up to 8 cycles of 3.7 GBq given \approx 6 monthly). Methods: Of 184 NET patients treated with Lu-177, 126 had <less than 6 cycles (9 withdrawn; 117 ongoing), 58 had \geq at least 6 cycles (6-7 cycles; n=27; 8-9; n=18; 10-11; n=13). In 58, the primary included: PNET (n=21), GNET (n= 27), likely GNET (n=3), other (n=7). Cumulative mean administered dose was 28.8 ± 4.5 GBq for 6-7 cycles, 35.7 ± 3.6 GBq for 8-9 and 44.5 ± 6.4 GBq for 10-11. Results: 43 39 patients remain on treatment. 15 19 patients have stopped treatment: 1 is disease free (after 6 cycles); 141 discontinued treatment due to biochemical, anatomic or symptomatic progression between cycles (after 6 cycles; n=97, after 7; n=1, after 8; n=21, after 10; n=2); 4 died (after 7 cycles; n=1, after 8; n=2, after 9; n=1). Lymphopenia (\geq (at least Grade 3, n=10) was the most common measurable adverse event (AE). Other Grade 3 AEs occurred in platelets (n=1), creatinine and eGFR (n=2), and creatinine (n=2). No myelodysplasia was seen. Conclusion: Induction and maintenance therapy with Lu-177 is a safe regime for NET patients. This regimen is potentially more effective than literature reported treatment regimens; in the cohort presented, median survival has not been reached at 54 months.

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Otolaryngology Head & Neck surgery

Surgery

Survival Benefit of Chemotherapy in Oropharyngeal Cancer Patients Treated with Surgery & Post-Operative Radiation

Han Zhang, Lakshmi Puttagunta, Daniel A. O'Connell, Jeffrey Harris, Hadi Seikaly and Vincent L. Biron

Purpose/Objectives: The benefit of chemotherapy in the post-surgical treatment of advanced stage oropharyngeal squamous cell carcinoma is unclear in the current literature. This study aims to compare patients treated with primary surgery followed by radiation or chemoradiation to determine whether the addition of chemotherapy has a significant survival advantage. We hypothesized that chemotherapy could have a survival advantage dependent on p16 and tobacco smoking history. **Materials/Methods:** Advanced stage OPSCC patients diagnosed and treated at a single tertiary cancer care centre between 1998-2009 were reviewed from a prospectively collected database. Patients who received primary surgery followed by either radiation (S+RT) or chemoradiation (S+CRT) were included in the study. A review of patient records was performed to obtain patient clinical characteristics as well as detailed pathologic, treatment and survival data. P16 status was obtained by immunohistochemistry of a tissue microarray of surgical tumor specimen. Comparative analyses of survival were performed between patients who received S+RT and S+CRT, stratified according to HPV/p16 status and tobacco smoking history. **Results:** 171 patients were treated with S+RT or S+CRT of which 138 had p16 typing and smoking status for inclusion in our analyses (S+CRT=67, S+RT=71). In patients treated with S+CRT, 73.5 % received cisplatin and 26.5 % received carboplatin. In p16 negative and p16 positive non-smoking patients, no significant differences in survival were seen between patients treated by S+RT vs S+CRT. In p16 positive smokers, patients treated with S+CRT had significantly higher 5-year disease specific survival (88.2 %) compared to S+RT (59.7 %). Multivariate analysis showed these survival differences regardless of patient covariates and factors. **Conclusions:** In advanced stage OPSCC treated with primary surgery and radiation, the addition of platinum-based chemotherapy may have

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improve disease specific survival in select patients. Further prospective trials that include p16 status would be recommended verify this hypothesis.

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Ashlee Matkin

Medicine and Dentistry

Surgery

Enhancing the Accuracy of Thyroid Fine Needle Aspirate Biopsies Using Droplet Digital PCR

Ashlee Matkin, Morris Kostiuk, Karina Currie, Daniel A. O'Connell, Hadi Seikaly, David W.J. Cote, Jeffrey Harris, and Vincent L. Biron

Approximately 25% of cytology reports from thyroid nodule fine needle aspiration biopsies (FNABs) are classified as indeterminate. Many patients with this result will undergo a diagnostic hemithyroidectomy, with up to 70% of cases found to be benign on final pathology. Enhancing the accuracy of FNABs could therefore reduce unnecessary thyroid surgery. Droplet digital PCR (ddPCR) has the potential to identify pathogenic mutations and aberrant gene expression indicative of thyroid carcinoma using a low concentration of nucleic acid. This study aims to determine if ddPCR is a reliable method of biomarker analysis in thyroid FNABs. Ultrasound-guided FNABs were prospectively collected from 102 patients presenting with thyroid nodules at a tertiary referral centre for head and neck surgery. Samples were stored in RNA-preserving reagent for later nucleic acid extraction. ddPCR was performed on samples using ≥ 1 ng of cDNA with the following HEX/FAM-labelled probes. Gene expression levels were measured relative to an internal control (EEF) for PTEN, PIK3CA, MET, CCND1, MK167, TSHR, PPAR-g, LGALS3 and EGFR. Mutational analysis was performed with comparison to wild type control for HRAS pG12V, HRAS Q61R, HRAS Q61K, NRAS Q61R, NRAS Q61K and BRAF V600E. The primary outcome measure compared genetic profiling results to standard cytology reports and final histopathology in patients who received surgery. Mutational and gene expression data was reliably obtained with small amounts of nucleic acid within 24 hours of receiving the sample. Mutations were identified in a number of FNAB specimen, including the samples that resulted in a diagnosis of malignancy. Gene expression profiles demonstrated a broad range of expression for all genes tested. The detection of biomarkers of thyroid cancer in FNABs, through the use of

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ddPCR, may enhance the accuracy of FNABS and has the potential to become a new diagnostic tool.

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Allison McNamara
Medicine and Dentistry
Oncology

Significance of Autophagy Regulators and Anti-Phospholipid Antibodies in Colorectal Cancer

Allison McNamara and Kristi Baker

Colorectal cancer (CRC) is a heterogeneous disease and one of the top three most common cancers affecting both men and women in Canada. Colorectal cancers (CRC) defective in mismatch repair genes exhibit high microsatellite instability (MSI-H) and consequently form many mutated proteins known as tumor associated antigens (TAA). MSI-H CRC are regarded as being more immunogenic than microsatellite stable CRC, however treatment is difficult due to the lack of conserved TAA between patients and a limited response to immunotherapy which is thought to be a consequence of the tolerant nature of the mucosal immune system. Exosomes released by CRC cells into the extracellular milieu by processes such as autophagy can carry TAA and display unique phospholipids (PPL) not typically found on the outer leaflet of the parental cell. These unique PPL can be bound by immunoglobulin G (IgG) to generate immune complexes (IC) and we hypothesize that engagement of the Fc region of the IC with Ig-binding receptors on dendritic cells can prime T-cell mediated CRC targeted immunity strong enough to overcome mucosal immunosuppression. Preliminary data demonstrates exosomes isolated from a murine CRC cell line (MC38) were identified by common exosome markers CD9, CD63, HSP-70, annexin V and exosomes from wildtype C57BL/6 mouse serum were highly bound by IgG. Additionally, mutations in autophagy regulators which degrade cellular proteins for antigen presentation have been identified in MSI-H CRC and could result in altered exosome composition making the exosomes more immune stimulatory or not. A screen of MC38 cells where different DNA repair genes were knocked out using CRISPR technology shows differential expression of autophagy related genes. We aim to further our preliminary data by examining these processes in cell lines, mouse models and human samples to better understand the pathogenesis of CRC and its relationship with the mucosal immune system.

Anais Medina Martin
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Smart Viral Nanoparticles for Molecular Targeting of Angiogenic Vasculature

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We hypothesize that molecular targeted viral nanoparticles (VNPs) based on TMV would specifically interact with tumor vasculature, leading to an early detection of tumor angiogenesis and occult metastatic disease. This study is aimed on the design and synthesis of an innovative dual TMV-based probe for in vivo optical and molecular imaging with positron emission tomography (PET). Tyrosine-based bioconjugation reactions were used to provide exquisite chemoselectivity to the 2130 exposed tyrosine residues per TMV molecule. Initially, we have focused on the coating of the VNPs with the fluorescent version of either polyethylene glycol polymers (PEG) or PEGylated peptide ligands targeting alpha v beta 3 integrin. In this regard, we have carried out the synthesis of the AlexaFluor647-labeled non-RGD integrin alpha v beta 3 targeting peptide (H-KTKKVHSQ-PEG3400-K(N3)-NH₂), which contains the polymeric chain as part of the sequence. The two polymers have been conjugated to the TMV via diazotization of the tyrosine on the surface of the VNP. In parallel, direct and prelabeling approaches were developed to radiolabel TMV with a 6-25% and 35-45% Cu-64 incorporation, respectively. We have established a toolbox of reliable modification and radiolabeling procedures for non-invasive multimodal imaging and exploration of TMV-based nanoparticles in vivo. First PET studies with unmodified TMV in PC-3-bearing tumor mice revealed minimal tumor uptake, while uptake was visualized in clearance organs, liver and kidneys, after 2h and 24 h p.i. Ultimately, the bi-PEGylated version of the TMV will be used to generate a targeted TMV that will also be subjected to radiolabeling with ⁶⁴Cu. This dual probe will be investigated in vitro and in vivo using the chicken chorioallantoic membrane (CAM) and prostate tumor bearing mice models. The replacement of ⁶⁴Cu with a therapeutic radionuclide such as ¹⁷⁷Lu will allow for the use of the TMV-based nanoparticles for prostate cancer treatment.

Daniel Nisakar Meenakshi Sundaram

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Targeting Integrin-beta1 to Reduce Attachment and Migration of Breast Cancer Cells

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Purpose: Cell surface integrins, which play important role in the survival, proliferation, migration and invasion of cancer cells, is a viable target for treatment of metastatic breast cancer. This line of therapy still remains challenging due to the lack of proper identification and validation of effective targets as well as the lack of suitable therapeutic agents for treatment. We have focused on one such molecular target for this purpose, namely integrin-beta1 and achieved effective lowering of integrin-beta1 levels on a breast cancer model (MDA-MB-231 cells) by delivering a dicer substrate siRNA targeting integrin-beta1 with lipid-modified low molecular weight polyethylenimine (PEI) polymers. Methods: Integrin-beta1 silencing experiments were carried out through Immunostaining, qRT-PCR, fibronectin-binding, human Bone Marrow Stromal Cell (hBMSC) adhesion, scratch and Transwell migration assay. Results: Three specific lipopolymers with different lipid substitutions displayed effective silencing at 40 nM siRNA concentration based on immunostaining of cell-surface integrin-beta1, but 1.2PEI-LA (with linoleic acid substitution) exhibited the best silencing among them. This treatment significantly silenced the cell surface level of integrin-beta1 and strongly silenced the mRNA levels till day 9. This helped to reduce its attach to fibronectin and human bone marrow stromal cells as well as the migration of MDA-MB-231 cells. Conclusions: Silencing integrin-beta1 gene in invasive MDA-MB-231 cells was shown to be feasible with a practical dose of specific siRNA. Both the fibronectin binding and hBMSC adhesion assay showed that the extent of integrin silencing at cell surface was adequate to reduce its binding and the Transwell as well as scratch assays showed reduction in their migration ability. In addition to silencing integrin-beta1, identifying other targets could be beneficial to fully reduce the attachment and migration of breast cancer cells thereby providing a viable method to specifically abolish an important mechanism behind breast cancer metastasis.

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Targeting the autotaxin-inflammatory axis to improve radiotherapy

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We established previously that adipose tissue adjacent to mouse breast tumors becomes inflamed by cytokines secreted from the tumor. This stimulates autotaxin secretion from adipocytes, whereas breast cancer cells produce insignificant autotaxin. Lysophosphatidate (LPA) produced by autotaxin promotes a vicious cycle of inflammation in adipose tissue and tumors by stimulating further secretion of inflammatory cytokines, which increase further ATX production. This inflammatory response promotes tumor growth, metastasis and resistance to chemotherapy. LPA also protects cells from radiation-induced cell death. Radiation treatment of breast tumors would inevitably expose adipose tissue at the margins of the tumor to radiation. We hypothesized that this should increase the secretion of autotaxin and other inflammatory mediators, which should protect the tumor against damage by further fractions of radiation. The present work tested the first part of this hypothesis using cultures of rat abdominal and human breast adipose tissue. Exposure to relatively low dose γ -radiation (0.25 to 1 Gy) increased mRNA concentrations for autotaxin, COX-2, IL-1 β , TNF α , IL-6, IL-10 and LPA1-3 receptors. There was also increased secretion of autotaxin and 14 cytokines and chemokines from human breast adipose tissue. Inhibiting the activity of PARP-1, NF κ B and COX-2 blocked the inflammatory response to γ -radiation. Consequently, collateral damage of adipose tissue at the margins of the breast tumor by radiation could establish an inflammatory/wound healing response, which could decrease the efficacy of subsequent fractions of radiotherapy.

Isaac Menghisteab

Science

Biological Science

TLR SIGNALING AND EXOSOME BIOGENESIS IN MICROSATELLITE INSTABLE
COLORECTAL CANCER

Isaac T. Menghisteab, Kristi Baker, Allison McNamara, Courtney Mowat, Sharmin S. Sumi

Most colorectal cancers have genomic instability in the form of chromosomal instability (CIN), some exhibit microsatellite instability (MSI). Germline mutation in mismatch repair genes such as MLH1 and MSH2 or sporadic silencing of these genes by hyper-methylation of the promoter region creates MSI. Notably, MSI-CRC prognoses are more favorable than CIN-CRC. Exosomes play a role in cancer through transfer of oncogenic protein, miRNA, and promoting angiogenesis and metastasis. Additionally, the colorectal environment is rich in microbes; toll-like receptor (TLR) signaling is activated by microbial products and induces changes in vesicle trafficking. We want to see what differences exist between MSI, and CIN, CRC and how they might shape the favorable prognosis of MSI-CRC. We hypothesized that MSI cells exhibit differential TLR signaling and vesicle trafficking patterns due to the breadth of involved genes which are prone to mutation in MSI cells. We compared the change in vesicle trafficking and exosome biogenesis in MSI and CIN cells following stimulation with TLR ligands. We used CRISPR knockouts of genes creating an MSI phenotype (MLH1, MSH2), and, ultramutable and CIN phenotypes. We used qPCR to quantify mRNA expression of genes involved in exosome biogenesis and vesicular trafficking. We observed greater expression of Rab27B and Vps28 in MODE-K CIN clones compared to MSI cells, and reduced expression of all genes in MODE-K clones compared to the empty vector. Reduced expression of Vps28 and Rab27B in MSI cells compared to CIN cells following TLR stimulation suggests MSI-CRC produce less exosomes than CIN-CRC. Analysis of protein level expression and exosome secretion will be used to confirm our findings. Given the cancer promoting roles of exosomes in cancer; reduced exosome biogenesis may contribute to the more favorable prognosis of MSI-CRC compared to CIN-CRC.

Anahita Mojiri

Medicine

Medicine

Functional assessment of Von Willebrand factor (VWF) expression by cancer cells of non-endothelial origin

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Introduction- Von Willebrand Factor (VWF) is an endothelial specific, adhesive, procoagulant molecule. Increased plasma levels of VWF and alterations in coagulation system in cancer patients with metastatic progression are reported. We hypothesized that some cancer cells with non-endothelial origin, express VWF, which facilitates their metastasis. Method- Several glioma and osteosarcoma cell types were analyzed for VWF expression using quantitative real time-PCR, western blot and immunofluorescence (IF) staining. Chromatin immunoprecipitation analyses were performed to determine association of VWF promoter with its regulatory transcription factors and its epigenetic modifications, in VWF expressing and non-expressing cancer cells. Adhesion and transmigration ability of VWF-expressing cancer cells were explored using FACS and Transwell assays. Extravasation ability of cancer cells was examined in Chick Chorioallantoic Membrane (CAM) assays and in a metastatic mouse model. Tumor patients' biopsies were analyzed for VWF-expressing cancer cells using IF staining. Results-De novo expression of VWF, at the RNA and protein levels, was demonstrated in several glioma and osteosarcoma SAOS2 cell lines. The pattern of transacting factors bindings and epigenetic modifications in VWF expressing cancer cells were similar to that in endothelial cells. Cancer cells expressing VWF demonstrated enhanced endothelial-adhesion and transmigration. Extravasation capacities of VWF-expressing cancer cells were significantly enhanced compared to those not expressing VWF. VWF knock down significantly reduced adhesion, transmigration and extravasation of VWF-expressing cancer cells, while over-expression of exogenous VWF in VWF non-expressing cancer cells resulted in their increased adhesion and extravasation capacities. VWF expressing cancer cells were

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detected in patient's tumor biopsies of glioma and osteosarcoma. Conclusion-De novo expression of VWF in cancer cells may enhance their metastatic potential.

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Hali Morrison

Medicine and Dentistry

Oncology

Preliminary Investigation into Collapsed-Cone based Dose Calculations for COMS Eye

Plaques

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Purpose: To investigate the accuracy of model-based dose calculations using a collapsed-cone algorithm for COMS eye plaques loaded with I-125 seeds. **Methods:** The Nucletron selectSeed 130.002 I-125 seed and the 12 mm COMS eye plaque were incorporated into a research version of the Oncentra Brachy v4.5 treatment planning system which uses the Advanced Collapsed-cone Engine (ACE) algorithm. Comparisons of TG-43 and high-accuracy ACE doses were performed for a single seed in a 30x30x30 cm³ water box, as well as with one seed in the central slot of the 12 mm COMS eye plaque. The doses along the plaque central axis (CAX) were used to calculate the carrier correction factor, T(r), and were compared to tabulated and MCNP6 simulated doses for both the SelectSeed and IsoAid IAI-125A seeds. **Results:** The ACE calculated dose for the single seed in water was on average within $0.62 \pm 2.2\%$ of the TG-43 dose, with the largest differences occurring near the end-welds. The ratio of ACE to TG-43 calculated doses along the CAX (T(r)) of the 12 mm COMS plaque for the selectSeed was within 3.0% of previously tabulated data, and within 2.9% of the MCNP6 simulated values. The IsoAid and SelectSeed T(r) values agreed within 0.3%.

Conclusions: Initial comparisons show good agreement between ACE and MC doses for a single seed in a 12 mm COMS eye plaque; more complicated scenarios are being investigated to determine the accuracy of this calculation method.

Courtney Mowat

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Experimental Oncology

Immune Consequences of Genetic Instability in Colorectal Cancer

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The increase in Colorectal Cancer (CRC) deaths is attributed to unhealthy lifestyles causing changes in the bacterial community of the gut and to gut morphology. A complication of CRC is the tolerogenic mucosal immune system, which controls the homeostatic bacterial microenvironment of the colon. Mucosal immune suppression hinders recognition of tumour-associated antigens, allowing tumours to establish before their detection. Approximately 80% of CRCs are chromosomally unstable (CIN), which is associated with a poor immune response, making it the deadliest form of CRC. In comparison, microsatellite instable (MSI) CRC only constitutes 15% of cases, but its enhanced immune response results in a better prognosis. Using CRISPR technology, we mutated genes specific to the mutation profiles of MSI and CIN CRCs. We hypothesize that mismatch repair (MMR) pathway mutations associated with MSI CRC cause aberrant proteins to build-up, enhancing the immune detection of cancer cells, and that this process is modulated by mutations in specific immune-associated genes depending on the type of DNA repair mechanism that is dysregulated. The goal of our research is to determine if non-MMR DNA repair pathway mutations alter bacterial recognition in a way that differentially impacts anti-tumour immune responses compared to MMR mutated cancers. We are currently focusing on the pattern recognition receptors (PRRs) of the innate immune system, which recognize pathogens and markers of DNA damage. We are stimulating our CRISPR'd cells with various PRR ligands, and measuring the change in expression of specific cytokines. Based on our hypothesis, we would expect cells with mutations emulating MSI or CIN CRCs to have an upregulation in the expression and activity of PRRs associated with protective immunity or destructive inflammation, respectively. If we can understand the immune consequences of genetic instability in colorectal cancer, then perhaps we can enhance the susceptibility of CRCs to available immune therapies.

Jihane Mriouah
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Oncology

One nanoparticle to deliver them all: development of the fusogenic liposomes platform for nucleic acid delivery.

Jihane Mriouah, Arun Raturi, Douglas Brown, Desmond Pink, Konstantin Stoletov, Clayton Bell, Deborah Sosnowski, John Lewis

The application of lipid nanoparticles for targeted delivery of therapeutic agents has gained considerable clinical acceptance in last decade. In particular, liposomes used as a nanocarrier in vivo improve the drug delivery, notably by stabilizing the therapeutic compounds and improving biodistribution. Moreover, liposomal delivery minimizes toxicity and immunogenicity. We have elaborated a nanocarrier platform for targeted drug delivery to prostate cancer leading to superior anti-tumor effects. The fusogenic liposomes are formulated with the transmembrane protein p14, allowing for the fusion of the liposomes with the membrane of target cells. Intracellular delivery is then independent of the endosomal pathway which represents an advantage for nucleic acid delivery. Consequently, we hypothesize that the fusogenic liposomes can improve the delivery of gene or small RNAs in vivo for cancer treatments. Although the liposomes produced by traditional manufacturing methods have been successfully used for drug delivery; low efficiency of encapsulation and number of poorly-scalable steps limit the possibility of large scale production in GMP setup for clinical trials. These methods also result in increased variability. To address these limitations, we are using the most advanced automated method for multi-scale nanoparticle production, offering high production reproducibility. We have optimized the formulation for nucleic acid delivery using precisely controlled microfluidics parameters. Our in vitro studies show that the resulting lipid nanoparticles deliver plasmid DNA to a similar efficiency to lipofectamine. Importantly, the properties of particles as well as the transfection efficiency are highly reproducible between batches and we have successfully scaled-up liposomal production from 250ul to 10ml. Using the chicken embryo model, we show that fusogenic liposomes successfully deliver plasmid DNA encoding for miR-122 in cancer cells in vivo, as a therapeutic approach to block

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metastasis. Finally, we are optimizing siRNA formulation of prostate-targeted fusogenic liposomes as an anti-metastatic treatment.

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Graeme Mulholland

Medicine

Surgery

Use of Droplet Digital PCR for Ultrasensitive Gene Expression Profiling and Mutational

Analysis of Salivary Gland Lesion FNA Biopsies

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Importance: Salivary gland tumors (SGT) represent a commonly occurring neoplasm within the head and neck. To date, preoperative diagnostic tools yield limited information to differentiate between benign and malignant lesions. Additional diagnostic tools are needed to more effectively treat these lesions. Objective: Fine needle aspirate biopsies (FNABs) of salivary gland tumors (SGT) have high diagnostic specificity but relatively low sensitivity. The recent discovery of biomarkers associated with distinct SGT provides the opportunity to enhance the pre-operative diagnostics. We aimed to utilize novel, highly sensitive technology termed droplet digital PCR (ddPCR) to identify diagnostic gene expression signatures from SGT FNAB samples. Design: Basic science description of epigenetic characteristics in SGT. Setting: Tertiary referral center for head and neck surgery. Patients and Methods: FNABs were pre-operatively collected from patients with SGTs and processed for ddPCR analysis. Gene expression levels were measured relative to internal control for EGFR, p53, Ki67, c-KIT, PTEN, PI3K, p16, MEK and fusion genes CRTC1–MAML2, MYB–NFIB, EWSR1–POU5F1, PLAG1-fusions and HMGA2-fusions. Expression profiles were correlated to post-surgical pathologic diagnosis. Results: FNAB samples from 48 patients with SGTs were collected for ddPCR analysis. Compared to other methods of biomarker analysis gene expression data was reliably obtained with small amounts of nucleic acid. Fusion gene products and distinct gene expression profiles were predictive of final surgical pathology. Conclusions: The detection of biomarkers of SGTs by ddPCR is a powerful diagnostic tool. The detection of altered gene expression and signature fusion-gene products associated with SGTs may be useful for pre-surgical planning.

Scott Murray

Medicine

Surgery

MODIFICATION OF THE SUBMANDIBULAR GLAND TRANSFER PROCEDURE

Scott Murray, Jeffson Chung, Han Zhang, Youness Elkhalidy, Hani Almarzouki, Naresh Jha, Rufus Scrimger, Brock Debenham, Vincent Biron, Daniel O'Connell, Jeffrey Harris and Hadi Seikaly

Importance: Adjuvant radiotherapy (RT) is an important part of treatment for advanced head and neck mucosal cancers. However, xerostomia remains a prevalent morbidity that significantly impairs patients' quality of life. The submandibular gland transfer has been shown to be superior in preventing radiation-induced xerostomia compared to pilocarpine in a phase III randomized control trial. However, its original description of transferring the gland into the submental area precludes its use in the oral cavity. Therefore, we developed the modified submandibular gland transfer (M-SGT) for use in oral cavity cancers where the submandibular gland contralateral to the disease process is transferred to the the peri-parotid space. Objective: To study the radiation sparing potential of the modified submandibular gland transfer. Design: Retrospective chart review Setting: Tertiary academic head & neck cancer center Participants: Head & neck cancer patients who have had surgery followed by radiation as their treatment and who have had a modified submandibular gland transfer Intervention(s) for Clinical Trials or Exposure(s) for observational studies: Modified submandibular gland transfer Main Outcome Measure(s): Radiation dose received by the transferred submandibular gland Results: We collected data on 16 patients who have had a modified submandibular gland transfer procedure performed by a single surgeon (HS). The mean radiation dose received by the transferred submandibular gland was 21 Gy (SD 14 Gy) which was significantly lower than the mean dose it would otherwise have received in the submandibular triangle, which was 54 Gy (SD 9 Gy, $p = 0.0001$). The received dose was well below the TD50 and maximum tolerable dose of the submandibular gland, which are 33 and 39 Gy respectively.

Marnie Newell

ALES

AFNS

Reduction of in vitro and in vivo growth of MDA-MB-231 breast cancer cells is amplified with docosahexaenoic acid in conjunction with doxorubicin through effects on cell cycle gene products

Marnie Newell, Vera Mazurak, Catherine J. Field

Docosahexaenoic acid (DHA) has been shown to reduce growth of breast cancer cells in vitro and in vivo. Doxorubicin (DOX) is a chemotherapy drug used in breast cancer treatment that has multiple actions in the cell including disruption of cell cycle. We have previously shown that MDA-MB-231 breast cancer cells pre-treated with DHA in vitro prior to DOX increases the cell killing activity of DOX. The objective of this study was to: 1) explore the effects on gene expression on DOX treated MDA-MB-231 cells pre-treated with DHA and 2) confirm that feeding DHA to nu/nu mice implanted with MDA-MB-231 tumours improves DOX treatment. . RNA was extracted for microarray analysis (Affymetrix GeneChip Human Gene 2.0) from MDA-MB-231 cells treated with DHA (60 μ M) in control medium (containing 40 μ M oleic acid / 40 μ M linoleic acid) or control media alone for 48h and then treated for 24h with DOX (4.1 $\times 10^{-7}$ M). In DHA+DOX cells compared to control, multiple genes specific to cell cycle were down-regulated (selection criterion of $p \leq 0.05$ and fold change ≥ 1.5), including Cyclin B1, PLK1, Wee1 and cdc25C. Protein analysis (Western blot) confirmed the changes in gene expression ($P < 0.05$). Nu/nu mice bearing MDA-MB-231 tumours were randomized to a high fat diet (20% w/w) \pm 5% w/w DHA diet (n=4/group) and treated IP 2 times/week with DOX (5mg/kg) for 5 weeks. Extracted tumours from the DHA+DOX diet group were significantly smaller than control (0.8 \pm 0.5g vs 2.3 \pm 0.8g). Immunohistochemistry staining and protein analysis of cell cycle proteins confirmed Wee1 and PLK1 were lower in DHA+DOX tumours ($P < 0.05$). These studies confirm that feeding a diet supplemented with DHA facilitates the effect of DOX on tumour growth and suggest that this may be occurring, at least in part, by amplifying the effect on the products of cell cycle specific genes. (supported by CIHR)

Sara Omar

Medicine and Dentistry

Oncology

In silico investigation of the structural differences between mutant and rescued p53 models

Sara Ibrahim Omar, Jack Tuszynski

The transcription factor, p53, plays a 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. Of the most frequent mutations in p53, are the single missense mutations at its DNA binding domain (DBD): R273H and R175H. The contact mutant, R273H-mutant p53 (mp53), harbors a mutation in a DNA binding residue. While R175 does not interact directly with DNA, R175H-mp53 is a structural mutant that leads to a conformational change in the DBD causing an indirect loss of DNA binding. Methylene quinuclidinone (MQ) binds covalently to p53 and restores the wild-type (wt) activity to mp53. Our aim is to investigate the structural effects of mutation on the binding of p53 to DNA and to investigate the effect of MQ binding on the mp53 structure. We built in silico atomistic models of the equilibrated R175H-mp53, R273H-mp53, MQ-R175H-mp53 and MQ-R273H-mp53, each in complex with DNA, and compared them to the wt-p53-DNA complex. Our results show that the residues fluctuation in R175H-mp53 is higher compared to wt-p53, which indicates a conformational change in the mutant. R273H-mp53 has a similar residue fluctuation pattern to wt-p53. The binding energies (BE) of mp53 to DNA, calculated using MMGBSA, demonstrate a marked increase in BE compared to wt-p53. Although, the binding of MQ to R175H-mp53 greatly decreases its BE to DNA, it does not have a significant effect on the BE of R273H-mp53. This suggests that MQ has a structural effect on mp53, which probably cannot make up for the lost electrostatic interaction between the DNA and the mutated positively charged R273 in R273H-p53. Therefore, its BE remains high. These results are critical for designing new p53 rescuers.

Igor Paiva

Pharmacy and Pharmaceutical Science

Pharmaceutical Science

Panitumumab-immunomicelles for actively targeting EGFR-overexpressing colorectal cancer cells

Igor Paiva, Mohammad Vakili, Michael Weinfeld and Afsaneh Lavasanifar.

High expression of epidermal growth factor receptor is intimately related to poor cancer prognosis and resistance to different therapies. Nanoparticles containing monoclonal antibodies on their surface have gained special attention for targeted and enhanced delivery of anti-cancer agents to EGFR overexpressing cancer cells. The long term objective of this research is to develop EGFR targeted nanoparticles for co-delivery of modulators of drug resistance plus anti-cancer agents to colorectal cancer cells. To this end, we have synthesized maleimide-poly(ethylene oxide)-b-poly(ϵ -caprolactone) (PEO-b-PCL). The ¹H-NMR spectrum confirmed the synthesis and the number average molecular weight of the di-block copolymer was 7,500 Da. Then, micelles were formed by self-assembly. Panitumumab (Vectibix®) was thiolated and attached to the functionalized surface of micelles using different antibody/polymer ratios (mol/mol) to make the immunomicelles (with EGFR antibody). The attachment was confirmed by native-PAGE and unreacted free antibodies were removed by size exclusion chromatography. The average micellar size before antibody attachment as measured by dynamic light scattering (DLS) was found to be 29.3±1.2 nm. This increased to 99.2±13.5, 100.3±4.4, 114.7±17.8, 81.9±14.2, and 99.3±10.5 nm for immunomicelles prepared using antibody/polymer ratios of 1/200, 1/100, 1/50, 1/25, and 1/10, respectively. Through micelle incubation with Cy5.5, the dye was physically loaded into the micelles. The fluorescently stained micelles were then used to measure the association of plain versus immunomicelles by HCT-116 (EGFR+) and SW-620 (EGFR-) cells using flow cytometry. The results showed a 1.24 fold increase in the association of immunomicelles with HCT-116 cells following 2 h incubation compared to plain micelles. In contrast, median fluorescent intensity remained similar when SW-620 cells were incubated with control and immunomicelles (P>0.05). The results showed feasible preparation of panitumumab-immunomicelles based on PEO-PCL which were more efficient in association with cells

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overexpressing EGFR. Therefore, the present nano-carrier is promising for targeted drug delivery to colorectal cancer.

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Robert Paproski

Medicine and Dentistry

Oncology

Using machine learning to identify aggressive prostate cancer using micro flow cytometry data

Robert J. Paproski, Desmond Pink, Deborah Sosnowski, Catalina Vasquez, John D. Lewis

Introduction: Men with circulating prostate-specific antigen (PSA) levels between 4-10 ng/ml typically receive prostate biopsies and most of these biopsies confirm no disease or tumors which do not require treatment. These biopsies are highly invasive and can cause lethal infections thus improved prognostic assays for prostate cancer aggressiveness are warranted. Circulating extracellular vesicles have generated interest recently due to their diagnostic and prognostic potential. We have developed a flow cytometry assay which can identify and enumerate submicron particles in the plasma which stain positive for two different biomarkers which are abundant in prostate tumors.

Methods: Pre-biopsy plasma samples from 224 men were stained for both biomarkers and analyzed with our assay. Flow data was processed into multiple regions of interest (ROI) based on large angle light scatter and fluorescence intensities of staining markers. Particle concentration in each ROI was analyzed using a variety of machine learning algorithms including decision trees, logistic regression, support vector machines, K-nearest neighbors, random forests, linear discriminant, and subspace discriminant analysis. Classifiers were created from the flow and clinical data (PSA, PSA density, previous biopsy results, digital rectal exam findings, and family history) using 5-fold cross validation to predict whether men had low or high aggressive disease determined by Gleason 3+4 and lower or Gleason 4+3 and higher biopsy results.

Results: Clinical data was moderately useful for determining cancer aggressiveness with a receiver operator characteristics area under the curve (AUC) of 0.73. Adding flow data from our assay increased the AUC to 0.83. The algorithms that provided the highest AUCs included support vector machines, linear discriminant and subspace discriminant analysis. Our results

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suggest that our flow assay significantly increases the ability to predict aggressive prostate cancer and may be useful for identifying which men should receive biopsies.

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Sindhuja Pattabhi Raman

Medicine & Dentistry

Oncology

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

Raman SP, Elsaidi H, Postovit LM, Weinfeld M, Kumar P.

Solid tumors have large areas with low levels of oxygen (known as hypoxic regions), and are associated with poor prognosis and treatment response. Tirapazamine (TPZ), a hypoxia targeting anticancer drug, started as a promising candidate to deal with this issue. However, it was withdrawn from the clinic due to severe neurotoxic side effects and impaired target delivery. Hypoxic cells overexpress GLUT transporters - a key feature during hypoxic tumor progression. My project aims at conjugating TPZ with glucose to exploit the upregulated GLUTs for its delivery, to facilitate both imaging and therapeutic management of hypoxic tumors. Glucose-conjugated TPZ (G-TPZ) will be selectively recruited to these receptors, facilitating its uptake in poorly oxygenated cells only. This targeted approach will minimize the toxicity to the oxygenated normal cells. Incorporating a radionuclide will further bestow both diagnostic and radiotherapeutic value to TPZ. Our hypothesis will be validated using selected head and neck and glioblastoma cancer cell lines, followed by its evaluation in pre-clinical animal models. I have already confirmed the overexpression of GLUT-1 in oxygen-depleted cells, which forms the basis for future studies with G-TPZ. Effects of TPZ therapy will be evaluated using key molecular markers of hypoxia such as the hypoxia selective transcription factor HIF1-alpha. The cytotoxicity of the conjugate will be determined by clonogenic and live/dead assays to evaluate its chemo/radiotherapeutic potential. Together this approach aims to overcome the limitations of TPZ therapy in the context of cancer treatment, and has a potential to transform TPZ into a multimodality theranostic (therapeutic+diagnostic) agent.

Faisal Rashed

Medicine and Dentistry

Oncology

Development of ACN: An Easier and Faster Alternative to Pimonidazole

Immunohistochemistry for Detecting and Mapping Molecular Hypoxia

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The hypoxic environment, a characteristic feature of solid tumours, is known to select and promote growth of a subpopulation of tumour cells with increased proliferative and metastatic potential. Widely regarded as a negative prognostic marker, high degrees of hypoxia are strongly correlated with low survival rates in cancer patients. Therefore, detection of hypoxia is crucial for making better treatment choices and for monitoring therapy responses. Nitroimidazoles have demonstrated a significant potential to evaluate hypoxia because of their ability to undergo selective entrapment in oxygen-depleted cells. Pimonidazole, a commercially available nitroimidazole derivative, is used in immunohistochemistry (IHC) to detect hypoxic regions in tissue biopsies. Here, we report the development of a novel molecule ACN, which offers an easier and faster alternative to Pimonidazole IHC. Our approach utilizes a copper catalyzed click chemistry technique that bypasses the necessity of using antibodies. Using a fluorophore attached alkyne, we have been able to confirm the hypoxia selective entrapment of ACN in cells as well as in xenograft tumour models. Our experiments demonstrated the co-localization of ACN and Pimonidazole, further strengthening our claim of ACN being equally efficient and significantly rapid in detecting hypoxia. Finally, utilizing a biotin labeled alkyne, we were also able to pull down and identify potential binding partners of ACN, which promises to provide mechanistic insights into its mode of action.

Andrej Roczkowsky
Medicine and Dentistry
Pediatrics and Pharmacology

Determining the role of intracellular MMP-2 activation in doxorubicin cardiotoxicity

Andrej Roczkowsky, Brandon Y.H. Chan, Bryan G. Hughes, Mathieu Poirier, Ramses Ilarraza, Richard Schulz

Doxorubicin is an effective anti-tumor drug used to treat a variety of cancers. Its therapeutic utility, however, is hindered because its major chronic side effect is dose-dependent myocardial injury. Doxorubicin cardiotoxicity is associated with increased oxidative stress, impaired calcium handling, and myofibrilysis in the heart. Oxidative stress directly activates matrix metalloproteinase-2 (MMP-2), an intra- and extra-cellular protease which targets sarcomeric and other intracellular proteins in the heart. We hypothesise that intracellular MMP-2 plays a role in doxorubicin cardiotoxicity by cleaving substrates in the sarcomere (troponin I) and sarcoplasmic reticulum (SERCA2a and phospholamban). Neonatal rat ventricular myocytes (NRVM) and human fibrosarcoma (HT1080) cells were treated with doxorubicin (0.5 μM) \pm selective MMP-2 inhibitors ARP-100 (1 μM) or ONO-4817 (1 μM) for 2-24 hr. Doxorubicin caused 15% cell death in neoplastic HT1080 cells but none in NRVM as measured by lactate dehydrogenase release. In NRVM, doxorubicin increased MMP-2 zymographic activity by 311% after 12 hr, which persisted at 24 hr. Increased MMP-2 activity was attenuated 67% with ARP-100 or ONO-4817. MMP-2 protein levels were increased 213% by 24 hr doxorubicin. Doxorubicin reduced protein levels of a confirmed MMP-2 target, troponin I, by 40%, which was not restored by ARP-100 or ONO-4817. Doxorubicin decreased phospholamban and SERCA2a protein levels by 44% and 39%, respectively, via a MMP-2 independent mechanism. Doxorubicin caused a 97% reduction in troponin I mRNA expression, trended to decrease phospholamban mRNA expression, and had no effect on SERCA2a mRNA expression, as measured by RT-qPCR. Doxorubicin at an antitumor concentration increases intracellular MMP-2 activity in cardiac myocytes. Doxorubicin decreases protein levels of troponin I possibly by reducing its mRNA expression. A limitation of this study was the use of neonatal cardiomyocytes which have an immature sarcomere and

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sarcoplasmic reticulum. Therefore, future studies should measure targets of doxorubicin-activated MMP-2 in adult cardiomyocytes.

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Ahmed Said

Medicine and Dentistry

Biochemistry

NEW APPROACH TO REDUCE COLORECTAL CANCER

Ahmed Said, Yahya Fiteih, Mohamed El Kalla, Shairaz Baksh

Introduction: Inflammatory bowel disease is a complex disease with unknown etiology which later in life may develop to colorectal cancer and it is highly prevalent in Canada. NOD2, pathogen recognition receptor protein, was the first gene identified in IBD patients in 2001. Upon NOD2 activation RIPK2, a member of receptor-interacting protein (RIP) family of serine/threonine protein kinases and a downstream adapter of the NOD2 signaling pathway, gets activated and regulates autophagy through ATG16L1 and NF κ B. RASSF1A or 1A is a tumour suppressor gene epigenetically silenced in human cancers and ulcerative colitis (UC) resulting in its functional inactivation. Previously we showed that 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. We also observed that *Rassf1a*^{-/-}*Nod2*^{-/-} showed higher survival rate and decreased disease severity upon DSS treatment. Which suggests that NOD2 pathway may be the driver of inflammation injury in DSS-treated *Rassf1a* mice. 1A forms robust association with *Nod2* upon muramyl dipeptide stimulation in colon cancer cells. We observed upon using 3-MA, autophagy inhibitor, on *Rassf1a*^{-/-} and *IL10*^{-/-} mice improve survival and also reduction in RIPK2 activity. RIPK2 activity was detected in colon snips from Ulcerative and Crohn's Disease patients through Immunohistochemistry. A RIPK2 inhibitor was processed by our lab and upon testing it in our mice model improve survival and reduced inflammation was observed. 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

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increased survival. Conclusion: Inhibition of inflammation and RIPK2 may be a new therapeutic option for treating IBD and to reduce the pre-disposition to colorectal cancer.

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Mohamed Salla

Medicine and Dentistry

Biochemistry

The role of RASSF1A in inflammation driven carcinogenesis and YAP signalling in the colon

Mohamed Salla, Marilyn Gordon, Natalia Volodko, Eytan Wine, Levinus A. Dieleman, Aduccio Thiesen and Shairaz Baksh

Recent priorities in cancer research are shifting to a better understanding of neoplasia promoting microenvironments and cancer development, spurring a much needed interest in preventative research and translation into pre-cancer interventions. A link between chronic inflammatory diseases (such as inflammatory bowel disease [IBD]) and the development of related cancers (such as colorectal cancer [CRC]) has long been recorded, but remains mechanistically poorly understood despite this being a promising target for malignancy-preventative interventions. Here we present mechanistic in vivo evidence demonstrating that chronic inflammation down-regulates the tumor-suppressor Ras-association domain family 1A (RASSF1A), with reciprocal up-regulation of the oncogenic RASSF1C isoform, increased tyrosine kinase activity, and dysregulation of the Hippo effector Yes-associated protein (YAP) resulting in invasive carcinoma. Active phosphotyrosine YAP complexes switch from YAP/p73 pro-apoptotic to YAP/TEAD and YAP/TBX3 pro-proliferative transcription. Upregulation of oncogenic RASSF1C, MYC, and DNA methyltransferases (DNMT) and activation of tyrosine kinases coincided with the appearance of neoplasia, suggesting that inhibition of YAP signaling is a promising new target to prevent IBD-CRC.

Asma Saqr

Pharmacy and Pharmaceutical Sciences

Pharmaceutical Sciences

Development of anti-CD20 immuno-micelles for active drug targeting to hematological cancers

Asma Saqr, Mohammad Reza Vakili, Raymond Lai, Afsaneh Lavasanifar

Development of anti-CD20 immuno-micelles for active drug targeting to hematological cancers Asma Saqr, Mohammad Reza Vakili, Raymond Lai, Afsaneh Lavasanifar Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada
Purpose: The aim of this study was to covalently attach the anti CD20 antibody, rituximab, to the surface of the polymeric micelles and assess the effectiveness of this approach in enhancing micellar specific interaction with target cells overexpressing CD20. Methods: Rituximab, was coupled to Cy5.5 NHS ester at pH=8. The Cy5.5 conjugated antibody was coupled to 3-(N-succinimidylxyglutaryl)aminopropyl, polyethyleneglycol-carbamyl distearoylphosphatidyl-ethanolamine (NHS-PEG-DSPE). Cy3-N3+ was covalently conjugated to a triblockcopolymer, methoxy poly(ethylene glycol)-b-poly(ϵ -caprolactone)-b-poly(α -propargyl carboxylate- ϵ -caprolactone) (MPEG-PCL-PPCL), through click reaction. Mixed micelles were then prepared by incubating Cy5.5 labeled antibody modified PEG-DSPE with MPEG-PCL-PPCL-Cy3 at 1:1 molar ratio overnight. Size, critical micellar concentration (CMC) and kinetic stability of mixed micelles (without any cy3 or cy5.5 probes) were measured by zetasizer (DLS measurement) and compared to that for micelles from the individual polymers. The size and morphology of micelles was also investigated by TEM. Flowcytometry was used to follow the association of plain versus antibody modified mixed micelles with CD20 over expressing PTLN cells using fluorescence at 570 and 707 nm, for Cy3 and Cy5.5, respectively. Results: The formation of mixed micelles was confirmed by DLS by detecting one peak around 65nm for mixed micelles compared to two separate peaks around 50.4 and 23.1 nm for micelles of individual polymers. Similar results were also found by TEM. The CMC of the mixed micelle was 7.8 μ g/mL; significantly lower than that of NHS-PEG-DSPE micelle (35.6 μ g/mL), and higher than that of MPEG-PCL micelle (3.05 μ g/mL). Kinetic stability of the mixed micelle was not significantly different from the MPEG-PCL

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micelle; however it was significantly higher than that of the NHS-PEG-DSPE micelles. Flowcytometry showed higher association of anti-CD20 micelles with PTLD cells compared to plain micelles and CD20 negative cells (SUP-M2). Conclusion: The results points to the effectiveness of PEG-DSPE/ MPEG-PCL mixed immune micelles modified on their surface with rituximab in enhancing their association with CD20 overexpressing cells.

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Katlyn Schiewe

Agricultural, Life and Environmental Sciences

Agricultural, Food and Nutritional Science

Breast cancer tumor growth reduced with dietary docosahexaenoic acid and chemotherapy

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Docosahexaenoic acid (DHA), a long chain polyunsaturated fatty acid found in cold water fish, has been shown to reduce breast cancer cell viability in vitro and in vivo. It has also been verified to increase cell death when used in conjunction with chemotherapy compared to chemotherapy treatment alone. The objective of this summer student project was to explore the effects of DHA when used prior to and during doxorubicin (DOX) chemotherapy treatment. Nu/nu mice (6 weeks old) were injected subcutaneously with MDA-MB-231 breast cancer cells (2×10^6) and fed a 20% fat w/w control diet ad libitum for four weeks. Tumour-bearing mice were then randomized to a diet (control or DHA 5% w/w, 2.2% of fatty acids) and were treated IP twice weekly with or without DOX (5mg/kg) for 5 weeks. Tumours were extracted, weighed and processed for lipid analysis, protein analysis, and immunohistochemistry. Final body weights of mice in the control+DOX group were significantly lower and the average tumor weight greater (2.3 ± 0.8 g vs 0.8 ± 0.5 g) than the DHA+DOX group ($p < 0.05$). Plasma and tumour lipids of mice fed a DHA diet were higher in DHA, compared to control ($P < 0.05$). Immunohistochemical analysis of tumor tissue found an increase staining of apoptotic markers (CD95 and TUNEL) in DHA+DOX, compared to control+DOX. Western blot analysis confirmed a higher content of apoptotic proteins (caspase 10, bid and Rip) in the DHA + DOX ($P < 0.05$). This study confirms that feeding a diet supplemented with DHA increases the effect of DOX chemotherapy on tumour growth. Our results suggest that DHA is playing a role in

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increasing CD95-mediated apoptosis in tumours. (K. Schiewe was funded by a summer student scholarship from AIHS, research funded by CIHR)

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Gabrielle Siegers

FoMD

Oncology

Gamma delta T cell Apoptosis Induced via “Blocking” Antibodies: A Cautionary Tale

Indrani Dutta, Dalam Ly, Lynne-Marie Postovit and Gabrielle M. Siegers

Mechanistic studies contribute greatly to our understanding of gamma delta T cell (GDTc) biology, aiding development of these cells as immunotherapeutic agents. The antibody blocking assay is an accepted method to determine the receptors involved in GDTc killing of tumour targets. Effectors and/or targets are pre-incubated with microgram quantities of so-called “blocking” antibodies, advertised as such by commercial sources. We and others have used such assays extensively in the past, correlating decreases in cytotoxicity against specific targets with involvement of the blocked receptor(s). However, we wondered whether other mechanisms might be at play beyond cytotoxicity inhibition. In a 4 hour cytotoxicity assay to assess GDTc killing of SUM149 breast cancer cells, blocking the gamma delta (gd)TCR reduced lysis of target cells by ~15%, and blocking with the Vdelta2 subset-specific antibody resulted in a two-fold reduction in %lysis. However, administration of the gdTCR blocking antibody on its own induced GDTc death, proportional to the observed decrease in cytotoxicity. Upon further investigation, we discovered that GDTc undergo apoptosis triggered by incubation with certain gdTCR “blocking” antibodies. Furthermore, the presence of interleukin (IL-)2 enhances this cell death. This induction of activation-induced cell death (AICD) also explains flow cytometry results in which we were unable to consistently detect blocking antibody binding to GDTc using fluorophore-conjugated secondary antibodies. The gdTCR undergoes rapid internalization upon stimulation with an activating antibody, thus it seems that some of these “blocking” antibodies may actually be “activating”. We have extended our studies to include proliferation assays and assessment of signaling pathways via Western blot analysis. Through testing a panel of purified anti-gdTCR antibodies, we aim to determine which clone(s) may prove better blocking antibodies than those currently in use.

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Sandeep Singhal

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Oncology

Role of Genomic Biomarkers (GBs) as predictors of response to cancer treatment

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Objective: The aim of this research was to demonstrate the gene expression changes with response to treatment. This information can be helpful to check the sensitivity of drug after short period of therapy. Specifically, to built a model to interrogate the association between neoadjuvant chemotherapy response (pCR in this case) and gene expression modules, recapitulating important biological processes such as proliferation, immune, stroma and "druggable" oncogenic pathways in different breast cancer subtypes. Patients and Methods: We searched for publicly available gene expression studies evaluating anthracycline with or without taxane-based neoadjuvant chemotherapy and identified eight studies with 996 patients. We computed 17 gene modules and calculated odds ratios (ORs) for pathologic complete response (pCR) for one-unit increases in scaled modules with and without adjustment for clinicopathologic characteristics. Added predictive accuracy was evaluated using the area under the receiver operating characteristic curve (AUC) and integrated discrimination index (IDI). We used the false discovery rate (FDR) to adjust for multiple testing. Results: We demonstrated different biological processes and pathways are associated with outcome in different BC subtypes.

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Priya Sivarajah

Faculty of Medicine

Department of Surgery

Depression as a predictor of postoperative functional performance status (PFPS) and treatment adherence in head and neck cancer patients: a prospective study

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Cote MD MPH CCFP FRCSC, Vincent Biron MD PhD FRCSC, Hadi Seikaly MD MAL FRCSC

Background Head and neck cancer (HNC) is a debilitating disease due in part to its effects on speech, swallowing, and cosmesis. The aim of this study was to assess the relationship between preoperative depressive symptoms (PDS) and postoperative functional performance status (PFPS), in addition to other predictors of rehabilitation and survival. Methods A prospective cohort study was undertaken at the University of Alberta, including all new adult HNC patients undergoing surgery as primary therapy for HNC from May 2013 to January 2014. Baseline depressive symptoms were measured on the Quick Inventory of Depressive Symptoms (QIDS) questionnaire 2 weeks preoperatively and PFPS was assessed 12 months postoperatively on the Functional Assessment of Cancer Therapy-Head & Neck FACT-HN) scale. Secondary outcomes included completion of adjuvant therapy, narcotic dependence, return to detrimental habits, loss of follow-up, and length of hospital stay (LOHS). Differences between the Normal-Mild and Moderate-Severe QIDS groups were assessed using Mann-Whitney and Fischer Exact statistical analyses. Survival to date was analyzed using Kaplan-Meier analysis. Results Seventy-one patients were included in the study. Mild and Moderate Severe PDS were 35.2 % and 18.3 %, respectively. Significantly lower FACT-HN scores were noted in the Moderate-Severe group at 12 months ($p=0.03$). The risk ratio (RR) for FACT-HN score <50 % at 12 months in the Moderate-Severe group was 5.66. In addition, significantly lower completion of adjuvant treatment ($p=0.03$), significantly higher incidence of narcotic dependence ($p=0.004$), and significantly higher LOHS (24 days vs. 18 days; $p=0.02$) was observed in the Moderate-Severe group. Survival was not significantly different between groups at approximately 18 months ($p=0.960$). Conclusions Patients with Moderate-Severe PDS have significantly decreased PFPS, increased narcotic use, decreased completion of adjuvant therapy, and a longer LOHS after surgical treatment for HNC.

Priya Sivarajah

Medicine

Surgery

Validation of Metabolic Tumour Volume as a Prognostic Factor for Oral Cavity Squamous Cell Carcinoma Treated with Primary Surgery

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Background Despite the promise of metabolic tumor volume (MTV) as a risk-stratifying marker, the retrospective design of the initial study limits its generalizability. Therefore, this study sought to validate MTV as a prognostic factor for oral cavity squamous cell carcinoma (OCSCC) treated with primary surgery within an independent data set. Methods The validation data set consisted of 42 patients diagnosed with OCSCC between 2008-2012. The original cohort consisted of 80 patients. MTV and SUVmax were calculated for the primary tumor and nodal metastasis separately, as well as combined. Before statistical analysis, MTV and SUVmax values were divided into intertertile thirds to allow for intergroup survival analysis. Validation analysis was conducted on the validation data set alone. Data from both cohorts were then combined (n=122) to increase statistical power. Results An increase in combined MTV of 17.5 cm³ was associated with statistically significant increase in risk of disease recurrence (HR = 19.2, p<0.001) and death (HR = 9.2, p<0.05). Combined SUVmax failed to predict overall (HR=1.0, p>0.05) and disease-free survival (HR=1.0, p>0.05). Increase in the MTV of the primary tumor was associated with an increase in the risk of disease recurrence (HR=21.7, p=0.0001) and risk of death (HR=7.0, p=0.0001), while increase in the MTV of the locoregional neck metastasis was not (p>0.05). An MTV cutoff value of greater than 10.2 cm³ was found to significantly affect survival. Conclusion Due to the reproducibility of MTV findings, this study validates MTV as an independent prognostic factor for OCSCC treated with primary surgery.

Hoda Soleymani Abyaneh
Pharmacy & Pharmaceutical Sciences
Pharmaceutical Sciences

Development of EGFR-targeted cisplatin micelles for the treatment of triple negative breast cancer

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Purpose: The objective of this study is to develop an EGFR-targeted polymeric micellar formulation of cisplatin for the treatment of triple negative breast cancer (TNBC). There has been a renewed interest in the use of cisplatin in TNBC; however development of cisplatin resistant is a significant clinical obstacle. In this study, EGFR-targeted nanoformulation of cisplatin was developed and the potential of this system for enhanced cisplatin delivery and thus activity under normoxia and hypoxia was investigated. Methods: Acetal-poly(ethylene oxide)-block-poly-(α -carboxyl- ϵ -caprolactone) (acetal-PEO-b-PCCL) micelles were prepared through ring opening polymerization of α -benzyl carboxylate- ϵ -caprolactone with acetal-PEO and followed by hydrogenation of benzyl carboxylate groups. Block copolymers self-assembled to micelles and modified with EGFR-targeting peptide, GE11 (YHWYGYTPQNVI) through Schiff base reaction. GE11 and plain micelles were characterized for their size, polydispersity, cisplatin encapsulation efficiency, and pH sensitive release behavior. The cisplatin cytotoxicity for plain and GE11 micellar formulations was investigated under both conditions using MTT, trypan blue, clonogenic and cell uptake assays. Results: Cisplatin micelles were 70-160 nm in size and released the drug slowly in PBS (pH=7.4). Cisplatin release was enhanced in acetate buffer saline (pH=5.0). GE11-micelles showed even a slower rate of release as compared to plain micelles in the latter media (12% and 20% of cisplatin content was released within 48h, respectively). MDA-MB-231 cells showed a high expression of EGFR protein under both normoxia and hypoxia. Cisplatin micelles showed lower cytotoxicity as compared to free drug under both conditions, suggesting the slow rate of drug release from micellar formulation. GE11-micelles revealed enhanced platinum cellular uptake as compared to plain micelles under hypoxic conditions (~2 fold). Conclusion: The results points to the potential of GE11 modified cisplatin micelles as

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delivery systems for cisplatin to TNBC cells. Modifications in the method of drug loading may be needed to ensure drug stability upon micelle incorporation.

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Hoda Soleymani Abyaneh

Pharmacy & Pharmaceutical Sciences

Pharmaceutical Sciences

The interplay of HIF-1 α and Myc under hypoxia promotes the generation of more tumorigenic and resistant phenotype of MDA-MB 231 cells

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Purpose: To elucidate the interplay of HIF-1 α and Myc under hypoxia in triple negative breast cancer (TNBC) cells. Methods: Two subsets of MDA-MB 231 cells sorted based on differential responsiveness to a Sox2 regulatory region (SRR2) reporter were used. The cell subset responsive to SRR2 reporter (RR cells) is significantly more tumorigenic than the reporter unresponsive (RU) cells. The effect of hypoxia (1% oxygen) on the conversion of RU to RR cells was investigated by measuring GFP expression and luciferase activity. Hypoxia induced expression of CD44⁺/CD24⁻ marker, chemoresistance to cisplatin and colony formation in RU, RR and Myc-overexpressing stable RU cells were also measured. Results: Hypoxia induced stem-like features in both RU and RR subsets, but to different extent. A small proportion of RU cells converted to RR cells under hypoxia. This coincided with moderately higher expression of stem cell markers in hypoxic RU cells. In contrast, RR cells exhibited higher expression of stem-like features, including a higher proportion of CD44⁺/CD24⁻ cells and chemoresistance to cisplatin under normoxic and particularly hypoxic conditions. Hypoxia induced upregulation of HIF-1 α , but potently suppressed c-Myc expression in both RU and RR cells, but the residual c-Myc in hypoxic RR cells was still much higher than that in hypoxic RU cells. Enforced expression of c-Myc in RU cells effectively conferred stem-like features particularly under hypoxia. Importantly, the level of stemness in hypoxic Myc-overexpressing RU cells was similar to that of RR cells. Conclusion: Hypoxia-induced HIF-1 α up-regulation leads to moderate acquisition of stem-like features in RU cells perhaps because of c-Myc suppression. RR cells on the other hand retain their higher stemness due to the higher residual c-Myc in hypoxia. Persistent expression of c-Myc is a bona fide marker of stemness in MDA-MB-231 cells, especially after hypoxia insult.

Connor Sommerfeld
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Efficacy of a High-Observation Protocol (HOP) in major head and neck cancer surgery: a prospective study

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Background: Head and neck cancer (HNC) is a complex diagnosis requiring intricate, multidisciplinary care. The aim of this study was to optimize an existing clinical care pathway with a High-Observation Protocol (HOP) to limit mechanical ventilation and sedation, and determine the effect on pulmonary morbidity, length of intensive care unit admission (LoICU), and hospital stay (LOHS). Methods: All HNC patients undergoing major ablative and reconstructive surgery as primary modality treatment from August 1, 2014, to May 31, 2015, at the University of Alberta were included in the study. Demographic, comorbid, and tumor characteristics for all patients were compared. The HOP mandated initiation of spontaneous breathing trials (SBTs) prior to the conclusion of the surgery, weaning of sedation, and limiting mechanical ventilation. Comparison was made to a historical cohort regarding LOHS, LoICU, readmissions to the intensive care unit (ICU), and incidence of pneumonia. Statistical analysis was completed using a Mann-Whitney analysis for continuous variables, and chi-squared analysis for discrete variables. Results: Ninety-six and 52 patients were observed in “Historical” and “Plateau” cohorts, respectively. No differences in demographic or tumor characteristics were observed between groups. LoICU (1.9 vs 1.2 days, $p=0.021$) and LOHS (20.3 vs 14.1 days, $p=0.020$) were significantly shorter in the “Plateau” cohort. ICU readmissions (10.4% vs 1.9%, $p=0.013$) were significantly less in the “Plateau” cohort. No significant difference was observed between groups regarding incidence of pneumonias. Conclusions: Rapid weaning of sedation and limiting mechanical ventilation may contribute to a shorter LoICU and LOHS, as well as decreased ICU readmissions.

Hilmar Strickfaden
Medicine Dentistry
Experimental Oncology

Multicolour Electron Spectroscopic Imaging (mESI) as tool to close the resolution gap

Hilmar Strickfaden, Christian Förster, Frank Wuest, Michael J. Hendzel

Tremendous progress in the development of new light-optical techniques has recently led to major break throughs in light microscopy (e.g. overcoming the longstanding limitation in optical resolution). However, there is still a gap between atomic resolution (NMR, x-ray crystallography) and light-optical superresolution microscopy that needs to be filled in order to visualize macromolecular complexes in-situ. We are trying to fill this gap by developing a set of new molecular probes for electron spectroscopic imaging (ESI) that can be used to generate multi-color pictures (like in fluorescence microscopy) of biologically relevant structures at a true nanometer scale.

Ryan Stubbins
University of Alberta
Internal Medicine

CD3 T-cell Infiltrates at Diagnosis Predicts Overall Survival in Solid Organ Transplant Recipients with Post-Transplant Lymphoproliferative Disorders (PTLD)

Stubbins, R.J., Lai, R., Preiksaitis, J., Zhu, J., Peters, A.

The host's immune status is central to both the pathogenesis and treatment of Post-Transplant Lymphoproliferative Disorders. (PTLD) We hypothesized that CD3 positive T-cell infiltrate density in the tumor microenvironment, a surrogate of the potency of the host versus tumor immune response, may be prognostic of overall survival in PTLT. A database consisting of 131 biopsy confirmed PTLT cases occurring in pediatric and adult solid organ transplant recipients after the year 2000 in Alberta were analyzed for clinical prognostic variables and overall survival. CD3 infiltrate was determined by a blinded pathologist (JZ) using an integer scoring system (0 - 3) to quantify CD3-positive cells in archived, formalin-fixed paraffin embedded tissue stained by immunohistochemistry. Tissue was available on 72 patients. Survival analysis was done by Cox regression, with between group differences tested by a

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Pearson's chi-square test, with $p < 0.05$ being taken as significant. Histology subtypes included early ($n = 7$), polymorphic ($n = 17$), monomorphic, ($n = 100$) Hodgkin, ($n = 8$) and unavailable. ($n = 2$) A denser CD3 T-cell infiltrate, defined as a CD3 score of 2-3, had a statistically significant protective effect by univariate Cox regression with respect to overall survival. (HR 0.352, $p = 0.008$) A CD3 score of 2-3 was negatively associated with a monomorphic histology ($p < 0.001$), but was not statistically associated with lymphocyte count, early PTLD, EBV status or bone marrow involvement. Multivariate Cox regression with respect to overall survival, including monomorphic histology, IPI 3-5, lymphocyte count < 1.0 and CD3 score of 2-3 again showed a statistically significant protective effect of a higher CD3 score. (HR 0.307, $p = 0.022$) A dense CD3 T-cell infiltrate in the tumor microenvironment at diagnosis is protective with regards to overall survival in PTLD by both univariate and multivariate Cox regression, versus clinical prognostic markers.

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Abu Talha
Dentistry
oral pathology

oral premalignancy potential change to malignancy

dr.abu talha

Oral premalignancy a potential change fundamental to malignancy Abstract: various oral mucosal lesions, particularly red lesions (erythroplasias) and some white lesions (leukoplakias), have a potential for malignant change. In general, the most common white lesions have the lowest risk of malignant transformation. Over 90% of malignant neoplasms (cancers) of the mouth are squamous cell carcinomas arising from mucosal epithelium. Approximately 500 cases of intraoral and lip carcinoma are registered each year in th BD. For the last 50 years the incidence of oral cancer has declined but, though it is rare, cases are now more frequently seen in those aged 30–50. Premalignancy is distinguished from malignancy only by the latter's invasiveness and release of metastases. Cancer of the mouth is considerably more common in men than women in most countries, and carcinoma of the lip is at least eight times as common in men as in women.the oral lesion may also be the relapse of carcinoma after chemotherapy ,radiotherapy and some times needs extensive surgery with reconstruction surgery. This cross sectional observational study was carried out in the oral and

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maxillofacial surgery department of Dhaka medical college from January 2015 to 2016
ianuary with the intention to know the prevalence of manifestation of premalignant and
malignant lesion and to help diagnosis and manage the disease in some extant. Cancer of the
mouth is considerably more common in men than women in most countries, and carcinoma of
the lip is at least eight times as common in men as in women .total 55 consecutive who were
already diagnosed among them 35 were premalignancy and 20 were malignancy with
hospitalized were evaluated as the period one year.different type of premalignant lesion and
malignant leion were found in our study population. Other finding include
malignant infiltration 10 cases. So an evaluation for underlying lesion should be considered in
various patient for improvement and correction and uses more valueable effective treatment
procedure in both disease states.

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Xiaoyun Tang

David Brindley

Biochemistry

Doxycycline attenuates breast cancer related inflammation by decreasing plasma
lysophosphatidate concentration and inhibiting NF-kB activation

Xiaoyun Tang, Xianyan Wang, Yuan Y. Zhao, Jonathan M. Curtis, David N. Brindley

We previously discovered that tetracyclines increase the expression of lipid phosphate
phosphatases at the surface of cells. These enzymes degrade circulating lysophosphatidate and
therefore doxycycline increases the turnover of plasma lysophosphatidate and decreases its
concentration. Extracellular lysophosphatidate signals through six G protein-coupled receptors
and it is a potent promoter of tumor growth, metastasis and chemo-resistance. These effects
depend partly on the stimulation of inflammation that lysophosphatidate produces. In this
work, we used a syngeneic orthotopic mouse model of breast cancer to determine the impact
of doxycycline on circulating lysophosphatidate concentrations and tumor growth.
Cytokine/chemokine concentrations in tumor tissue and plasma were measured by
multiplexing laser bead technology. Leukocyte infiltration in tumors was analyzed by
immunohistochemistry. The expression of IL-6 in breast cancer cell lines was determined by
RT-PCR. Cell growth was measured in Matrigel™ 3D culture. The effects of doxycycline on
NF-kB-dependent signaling were analyzed by Western blotting. Doxycycline decreased

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plasma lysophosphatidate concentrations, delayed tumor growth and decreased the concentrations of several cytokines/chemokines (IL-1b, IL-6, IL-9, CCL2, CCL11, CXCL1, CXCL2, CXCL9, G-CSF, LIF, VEGF) in the tumor. These results were compatible with the effects of doxycycline in decreasing the numbers of F4/80+ macrophages and CD31+ blood vessel endothelial cells in the tumor. Doxycycline also decreased the lysophosphatidate-induced growth of breast cancer cells in three-dimensional culture. Treatment of breast cancer cells with doxycycline also decreased the translocation of NF-kB to the nucleus and the mRNA levels for IL-6 in the presence or absence of lysophosphatidate. These results contribute a new dimension for understanding the anti-inflammatory effects of tetracyclines, which make them potential candidates for adjuvant therapy of cancers and other inflammatory diseases.

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Michael Taylor

Medicine

Retrospective review of recurrent rectal adenocarcinoma: a provincial audit

Michael Taylor, Donald Buie, Todd McMullen

Background: Approximately 500 patients are diagnosed with rectal cancer in Alberta each year. Most (~80%) receive surgery, representing ~5% of all cancer surgeries in Alberta. Previous studies of clinical outcomes following curative surgical resection for rectal adenocarcinomas (such as disease-free/overall survival (DFS/OS) or local/distal recurrence rates (LR/DR)) have been zone-specific or are outdated; consequently, accurate recurrence rates for the province have not been established. Recurrent rectal cancer presents a significant challenge to both patients and healthcare teams; curative options are limited and mortality can be quite high. Although patients receive good care overall in Alberta, there are gaps in care that contribute to known recurrence rates (i.e. 7.4% in Edmonton).

Objective: In order to effectively improve Albertan clinical practice patterns, this study aims to provide provincial disease-free/overall survival and local/distal recurrence rates as part of the Alberta Rectal Cancer Clinical Pathway Initiative (ARCCPI).

Methods: Patients diagnosed with Stage I-IV rectal cancer from January 1st to December 31st,

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2011 were identified from the Alberta Cancer Registry (ACR). A retrospective chart review identified if resections were performed, as well as resection dates. Consultation and progress notes, diagnostic imaging reports, operative reports, and pathology reports were mined for explicit mention of local or distal recurrences. Vital status (alive or dead) was obtained from the ACR on December 20th, 2015.

Results: 370 cases were included in the study (those who received curative resections for rectal cancer). 4-year LR (7.03%, 26/370) and 4-year DR (23.8%, 88/370) were calculated. 4-year OS was determined for all cases (75.7%, 280/370) and those with local recurrences (57.7%, 15/26). Total 4-year DFS was noted as 66.0% (244/370).

Conclusions: The current audit will chiefly inform the economic evaluation of the ARCCPI, as well as operations within AHS of the results from past practice patterns; this will aid in the subsequent approach to addressing gaps in care that may be preventing a provincial LR rate of 4%. Overall, as part of the ARCCPI, training and monitoring may be required to ensure appropriate surgeries are being performed across Alberta. The ability to monitor adherence through measurement of process and outcome measures is critical.

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Juliana Valencia Serna

Medicine

Biomedical Engineering

Targeting BCR-ABL fusion oncogene by siRNA lipoplexes in primary Chronic Myeloid

Leukemia cells

Juliana Valencia-Serna, Cezary Kucharski, Remant KC, Josep Brandwein, Xiaoyan Jiang, Hasan Uludag

Chronic Myeloid Leukemia (CML) is a malignant neoplasm characterized by the translocation of chromosomes 9 and 22 that gives rise to the BCR-ABL fusion oncogene at the myeloid hematopoietic stem cell level. BCR-ABL initiates and propagates the disease by activating pro-survival and anti-apoptotic signals that lead to an eventual expansion of immature myeloid cells in the marrow and bloodstream. Tyrosine kinase inhibitors (TKI) against the ABL

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tyrosine kinase has shown promise in treating CML patients, however some patients show early relapse and develop resistance to TKIs often due to mutations in the BCR-ABL kinase domain. Small interference RNA (siRNA) molecules can be used to trigger the RNA interference (RNAi) cellular mechanism to induce silencing of a particular overexpressing/aberrant gene for therapeutic effects. The application of this therapy however, relies on the use of a carrier for siRNA delivery into the cells. This study explores the effects of BCR-ABL downregulation by siRNA on the survival of CML primary cells using lipid-substituted polyethyleneimine (PEI) lipopolymers as siRNA carriers. An array of lipopolymers was screened for FAM-labelled siRNA delivery by flow cytometry in frozen and fresh CML patient samples (n=6). Internalization of siRNA molecules with most potential lipopolymers was analyzed and confirmed by TEM. Treatment of primary CML stem/progenitor cells (n=3) with BCR-ABL siRNA lipoplexes reduced BCR-ABL mRNA expression by 30-50%. siRNA-mediated suppression of BCR-ABL impaired significantly the ability of CML stem cells to form colonies in vitro. This study showed a successful method to efficiently silence BCR-ABL function by means of a siRNAs lipopolymer delivery carrier. This system may be a therapeutic agent with clinical relevance for an alternative treatment for CML.

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Kevin Vo

Medicine and Dentistry

Oncology

Understanding The NFI-Calpain Signaling Pathway in Malignant Glioma

Kevin Vo, Rebecca Burchett, Miranda Brun, and Roseline Godbout

Introduction: Malignant gliomas (MG) are the deadliest brain tumours because of their infiltrative nature. Nuclear factor I (NFIA, NFIB, NFIC, and NFIX) has been implicated in the regulation of genes involved in MG cell infiltration. NFI has been shown to be dephosphorylated and activated by calcineurin phosphatase whose activity, in turn, is regulated by calpain proteases. Interestingly, the CAST gene encoding calpastatin – a calpain endogenous inhibitor – is a putative target of NFI. The main objective of this project is to characterize the NFI-calpain signaling pathway and examine its effects on MG cell growth properties. Methods: Putative NFI binding sites in CAST were identified using a bioinformatics approach. Physical interaction between NFI and CAST was confirmed with gel

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shift assays and chromatin immunoprecipitation. RT-PCR was used to examine changes in CAST and calpain RNA levels in response to NFI depletion. Western blotting was used to investigate changes in calpastatin and calpain protein levels when NFI is overexpressed or knocked-down. Results: CAST is bound by NFIC and NFIX. NFI depletion, specifically NFIC and/or NFIX, results in increased CAST RNA levels, but does not alter calpain RNA levels. Protein analysis indicates that calpastatin expression is downregulated when NFIs are overexpressed in NFI-inactive (T98) cells and is upregulated when NFIs are depleted in NFI-active (U251) cells. Although NFI depletion does not affect calpain RNA levels, it causes a reduction in calpain proteolytic activity. Furthermore, MG cells exhibit a concentration-dependent reduction in migration and survival in response to calpain inhibitors. Conclusions: NFI regulates CAST gene expression. Changes in calpastatin and calpain levels upon altering NFI levels suggest a NFI-calpain positive feedback loop in MG cells. This regulatory loop may result in increased NFI and calpain activity, thereby enhancing MG cell migration and survival. Our data indicate that calpain antagonists may have therapeutic effects on MG.

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Larissa Vos

Alberta Health Services

Clinical Trials Unit

Beta Blockers and Improved Progression Free Survival in Patients with Advanced HER2

Negative Breast Cancer: a Retrospective Analysis of the ROSE/TRIO-012 Study.

Gonzalo Spera, Rodrigo Fresco, Larissa Vos, Helena Fung, Jason R. B. Dyck, Edith Pituskin, Ian Paterson, John R. Mackey

Purpose: Recent retrospective studies suggest that beta-adrenergic blocking drugs (BB) are associated with improved survival in patients with a range of cancers. Although limited and discordant data suggest that BB may increase overall survival (OS) of patients with localized breast cancer (BC), there is no information on the effects of BB in women with advanced BC. Patients and Methods: To explore the association between BB use and BC outcomes, we retrospectively reviewed data from the ROSE/TRIO-012 double-blinded, multinational phase III trial that randomized 1,144 patients with HER2-negative advanced BC to first-line treatment with docetaxel in combination with ramucirumab or placebo. We compared progression-free survival (PFS), OS, Overall Response Rate (ORR), and Clinical Benefit Rate

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(CBR) in patients who received BB to those who did not. Results: 153/1,144 (13%) patients received BB; 62% prior to enrolment and 38% began after enrolment. Median PFS in BB treated patients was longer in patients who did not receive them (10.3 vs. 8.3 months; HR 0.81; 95% CI 0.66-0.99; p=0.0379). Patients treated with BB only after enrolment had even higher median PFS (15.5 vs. 8.3 months, p=0.0005). In the TNBC subset, median PFS was 13 months with BB, compared to 5.2 months without BB (HR 0.52; 95% CI 0.34-0.79; p=0.002). The benefit of BB intake in PFS was independent of treatment-emergent hypertension (p=0.476) but dependent on treatment arm (p=0.037). The test for interactions between BB and treatment arm was not significant (p=0.276). No differences were seen in OS, ORR, or CBR. Conclusions: In this exploratory post-hoc analysis BB intake was associated with significant improvement in PFS, particularly patients with TNBC and patients not previously exposed to BB.

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Yixiong Wang

Faculty of Medicine and Dentistry

Experimental Oncology

Role of DEAD Box 1 Protein in Alternative Splicing Mediated by the Microprocessor

Yixiong (Jack) Wang, Lei Li and Roseline Godbout

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. It has previously been shown that the microprocessor 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 microprocessor complex. We hypothesize that DDX1 functions as an enhancer for the microprocessor complex to compete with the spliceosome complex, thereby altering the alternative splicing of a subset of pre-mRNAs. We have carried out gene expression microarray analysis of DDX1-depleted HeLa cells, and identified 'apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3H' (APOBEC3H) as the most differentially expressed gene. As the APOBEC3H gene has 4 splice known variants (variants 1 - 4), we selected this gene for further analysis. Upon transfection of HeLa cells with siRNAs targeting either DDX1 or DROSHA (a key component of the microprocessor complex), we observed a

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reduction in APOBEC3H variant 4 and an increase in APOBEC3H variant 1 compared to cells transfected with scrambled siRNA. This effect on APOBEC3H variants was enhanced when HeLa cells were transfected with DROSHA siRNA. These data suggest that DDX1 may indeed play a role in APOBEC3H alternative splicing by enhancing the activity of the microprocessor complex. We are currently pursuing these experiments by RNA-IP in order to further understand how DDX1 interacts with the microprocessor complex, thus shedding light on DDX1's role in alternative splicing.

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Ping Wee

FoMD

Department of Medical Genetics

EGFR endocytosis during mitosis is mediated primarily by CBL

Ping Wee and Zhixiang Wang

Background: Abnormal sustained epidermal growth factor receptor (EGFR) activity can lead to oncogenic cell cycle progression. Cells downregulate the levels of active EGFR by internalizing the receptors into the plasma membrane. Two distinct forms of EGFR internalizations exist: clathrin-mediated endocytosis (CME) and non-clathrin mediated endocytosis (NCE). NCE has been shown to depend on EGFR ubiquitination, most prominently induced by the E3 ligase c-CBL. Purpose: Whereas EGFR endocytosis has been intensely studied in interphase cells, the mechanism of EGFR endocytosis during mitosis has been poorly studied. We previously showed that the kinetics of EGF-induced EGFR endocytosis are different during mitosis. Here, we study the molecular mechanisms of EGFR endocytosis during mitosis, and show that it is mostly dependant on CBL-mediated NCE. Methods: EGFR endocytosis was induced by stimulating cells with EGF and observed by indirect immunofluorescence. High EGF dose of 50ng/mL (activates both CME and NCE) was used, unless mentioned otherwise. Results: Knockdown of c-CBL in HeLa cells caused a major decrease in EGFR endocytosis in mitotic cells, but not in interphase cells. Transfection of EGFR with mutations that prevent direct and indirect CBL-binding (Y1045F and 1045-truncation respectively) into MCF-7 and 293T cells also showed reduced mitotic endocytosis. EGFR ubiquitination assay showed that the EGFR was more heavily ubiquitinated during mitosis than during interphase. Transfection of HeLa cells with 70z-CBL (cannot ubiquitinate

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EGFR) decreased mitotic EGFR endocytosis, whereas overexpression of c-CBL increased mitotic EGFR endocytosis. Low dose EGF stimulation (2 ng/mL, only activates CME) caused low levels of mitotic EGFR endocytosis compared to interphase. Significance: Oncogenic EGFR may escape downregulation by entering mitosis. Furthermore, loss of CBL further exacerbates this. As cancer cells with oncogenic EGFR undergo mitosis more often than normal cells, elucidating the differences between interphase and mitotic EGFR endocytosis could lead to new avenues for targeting these cancers.

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Lian Willetts

Medicine and Dentistry

Oncology

Intravital identification of miRNA drivers of human cancer metastasis

Lian Willetts, Konstantin Stoletov, Juan Jovel, Emma Woolner, John Lewis

Metastasis is the leading cause of cancer patients' death. MicroRNAs (miRNAs) have been implicated as key regulators and biomarkers of cancer metastasis. Currently there is no prognostic biomarker signature and treatment regimen specifically targeting metastasis. We conducted the first in vivo whole human miRNAome screen for miRNAs that drives a critical rate-limiting step of metastasis: invasive cell migration. Several miRNAs that block the formation of invasive metastatic lesions were identified. Our preliminary miRNA target analysis revealed that these metastasis-regulating miRNAs have overlapping putative gene targets in the integrin and Rho GTPases driven signaling pathways. Specifically, target analysis using the DIANA and TargetScan tools indicated an enrichment of genes that mediate interactions with ECM: cell surface receptor kinases and ECM components, as well as small regulatory proteins. Furthermore, we found that some of the miRNAs in our screen act via interfering with the cancer cell's ability to productively remodel and engage to components of the extracellular matrix, disrupting directional cell migration along structures such as vasculatures aligned collagen bundles. Intravital image analysis revealed that cancer cells that are deficient in pro-metastatic miRNA expression fail to attach to vascular wall and invade along the vessel aligned collagen bundles. These findings were further confirmed by Affymetrix microarray analysis for selected miRNA screen hits. We found significant changes in expression of adhesion and migration related gene networks such as cytoskeleton

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regulation, focal adhesion, MAPK signaling and several other cell adhesion related genes. Since our screen identified miRNAs that are functionally involved in metastasis, we propose that they can serve as both biomarkers to predict metastasis and as therapeutic targets to block metastasis. We plan to continue these studies by comprehensive mapping the mRNA target network of screen-identified miRNAs and evaluating their therapeutic potential as inhibitors of directional cancer cell invasion.

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Chengsheng Wu

Faculty of Medicine and Dentistry

Lab Medicine and Pathology

A positive feedback loop involving the Wnt/ β -catenin/MYC/Sox2 axis defines a highly tumorigenic cell subpopulation in ALK-positive anaplastic large cell lymphoma

Chengsheng Wu, Hai-Feng Zhang, Nidhi Gupta, Abdulraheem Alshareef, Qian Wang, Yung-Hsing Huang, Jamie T. Lewis, Donna N. Douglas, Norman M. Kneteman and Raymond Lai

We have previously described the existence of two phenotypically distinct cell subsets in ALK-positive anaplastic large cell lymphoma (ALK+ALCL) based on their differential responsiveness to a Sox2 reporter (SRR2), with reporter responsive (RR) cells being more tumorigenic and chemoresistant than reporter unresponsive (RU) cells. To understand how the RU/RR dichotomy is regulated, we performed bioinformatics analysis of SRR2 and identified MYC as a potential regulator. In support of its role, MYC was highly expressed in RR cells compared to RU cells, and inhibition of MYC substantially decreased the Sox2/SRR2 binding, Sox2 transcriptional activity, chemoresistance and methylcellulose colony formation. In contrast, enforced expression of MYC in RU cells conferred the RR phenotype. The Wnt/ β -catenin pathway, a positive regulator of MYC, was highly active in RR but not RU cells. While inhibition of this pathway in RR cells substantially decreased MYC expression and SRR2 reporter activity, experimental activation of this pathway led to the opposite effects in RU cells. Collectively, our results support a model in which a positive feedback loop involving Wnt/ β -catenin/MYC and Sox2 contributes to the RR phenotype. In a mouse xenograft model, RU cells stably transfected with MYC showed upregulation of the Wnt/ β -catenin/MYC/Sox2 axis and increased tumorigenicity. Correlating with these findings, there was a significant correlation between the expression of active β -catenin and MYC in ALK+ALCL primary

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tumor cells. In conclusion, a positive feedback loop involving the Wnt/ β -catenin/MYC/Sox2 axis defines a highly tumorigenic cell subset in ALK+ALCL.

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Min Hsuan Wu

Medicine and Dentistry

Oncology

When damaged, cancer cells change their Sox2!

Min-Hsuan Wu, Amirali Bukhari, Armin Gamper

Cancer is the leading cause of death in Canada and current treatment options such as radiation and chemotherapy attempt to induce lethal levels of DNA damage in tumour cells. Within a tumour, only a subset of cells are able to initiate neoplasms when transplanted or following localization to different organs. These tumour-initiating cells, often called Cancer Stem Cells (CSCs) because of their high cellular plasticity, were found to exhibit particular resistance to genotoxic therapies. They are also thought to be the primary cause of local recurrence and metastasis. These CSCs display characteristics similar to that of induced-pluripotent stem cells, namely in their ability to self-renew and potential to differentiate into different cell types. My project focuses on a transcription factor involved in the induction of dedifferentiation, Sex-determining region Y-box protein 2 (Sox2). Sox2 is an essential gene crucial during normal embryonic development and adult stem cell renewal. Moreover, its amplification is detected in multiple cancers. As Sox2 has been linked to a more invasive phenotype, aberrant expression observed in cancer may contribute to the stem-like phenotypes of CSCs. Based on the hypothesis that there is an interaction between the DNA damage response and stem cell renewal signalling, my project will investigate the effect of Ionizing Radiation (IR) and DNA damaging drugs on the regulation of Sox2. The regulation of Sox2 following DNA damage remains as yet uncharacterized, however, my preliminary studies show that Sox2 levels and activity are regulated by DNA damage signalling in several cancer cell lines. I intend to study the specific upstream signalling pathways regulating Sox2 levels following genotoxic stress and the control mechanisms of its activity. Thereby, we hope to not only gain insight into mechanisms underlying CSC plasticity, but also establish whether changes in Sox2 activity affects cancer cell fate.

Xia Xu

Medicine and Dentistry

Oncology

Investigate role of DHA in malignant glioma

Xia Xu; Roseline Godbout

Malignant glioma (MG) is the most common primary malignancy in brain. Brain fatty acid binding protein (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 (PPAR γ), a transcription factor with tumour suppression function involved in regulation of lipid metabolism and cancer cell migration. DHA treatment has been shown to inhibit MG cell growth and migration in vitro. This study was designed to investigate whether DHA plays an inhibitory role in MG cell migration through FABP7-mediated nuclear transportation with subsequent transcription factor activation. We used stable U87 control and U87 FABP7-transfected MG cell lines as models to test the effect of FABP7 and DHA on gene expression. Cells were treated with bovine serum albumin (BSA) vehicle, 60 μ M AA or 60 μ M DHA for 24 hours. To date, we have shown that both U87 control and U87 FABP7-transfected cells express elevated levels of STAT3 and phosphorylated STAT3 (pSTAT3). DHA supplementation downregulates pSTAT3 (activated form of STAT3), possibly through FABP7-mediated nuclear transport of DHA. Our previous data indicate that DHA-induced inhibition of migration in U87 FABP7 cells is partially through PPAR γ . Furthermore, knockdown of PPAR γ in FABP7-positive but non-migratory M049 malignant glioma cells results in increased migration. Our results to date suggest that DHA-induced migration inhibition in malignant glioma cells is mediated through activation of STAT3a and expression of PPAR γ . We will pursue our analyses by investigating the effect of FABP7 and fatty acids on other nuclear receptors and lipid metabolism proteins, and examining the effect of DHA and FABP7 on cell proliferation, neurosphere formation and tumour formation.

Zelei Yang

Medicine and dentistry

Biochemistry

Role of autotaxin and inflamed mammary adipose tissue in the development of doxorubicin resistance in breast cancer

Zelei Yang, Ganesh Venkatraman, Xiaoyun Tang, Matthew GK Benesch, Amadeo M Parissenti, Todd PW McMullen, Denise Hemmings and David N Brindley

Autotaxin promotes breast tumor growth, metastasis and chemo-resistance. Breast cancer cells express little autotaxin, but secrete inflammatory cytokines that stimulating autotaxin secretion from surrounding adipose tissue. Autotaxin produces lysophosphatidate (LPA), which results in a vicious inflammatory cycle that is a part of the wound healing response. This is hijacked by tumors (wounds that do not heal). We hypothesized that damage of breast tumors during doxorubicin therapy would elicit this wound healing response, which would decrease the efficacy of the therapy. We treated human MCF-7 breast cancer cells or human adipose tissues with combinations of LPA and doxorubicin and then analyzed the production of inflammatory mediators using qRT-PCR and multiplex ELISA. We also used a syngeneic mouse model with 4T1 breast cancer cell injection into the mammary fat pad. We injected doxorubicin or PBS every third day throughout tumor development. The expression of inflammatory mediators in the breast tumor and in adjacent and contralateral adipose tissue were analyzed. MCF-7 cells selected for resistant to doxorubicin show higher expression of inflammatory cytokines. Treatment of MCF-7 cells with LPA amplified the doxorubicin-induced expression of inflammatory cytokines. In adipose tissue, autotaxin expression increases when treated with LPA in the presence of doxorubicin. Mice treated with doxorubicin show increased expression of inflammatory mediators in the adipose tissue adjacent to the breast tumor, but not in the contralateral fat pad. Our results support the hypothesis that damage caused by doxorubicin establishes an inflammatory cycle of cytokine and autotaxin secretion. This response attempts to heal damage caused by the chemotherapy, which therefore decreases the efficacy of chemotherapy. Our results provide an additional understanding for the development of acquired chemo-resistance. We propose that blocking LPA signaling could provide an adjuvant therapy to increase the efficacy of chemotherapy.

Wan Kong Yip

Faculty of Medicine & Dentistry

Department of Medical Microbiology & Immunology

Characterization of Reovirus Mutant with Improved Replication and Dissemination in Cancer Cells

Wan Kong Yip, Georgi Trifonov, Nashae Narayan, Mark Kubanski and Maya Shmulevitz

Mammalian reovirus is a non-pathogenic virus evolved to infect the gastrointestinal and respiratory tracts. T3wt (wild-type) selectively replicates in cancer cells and it is being evaluated in more than 30 completed and ongoing clinical trials internationally. We aimed to improve oncolytic potency of reovirus by screening for variants with enhanced replication in various mouse and human cancer cells. Two variants, T3v1 and T3v2, were found to have improved onset of infection due to decreased levels of cell-attachment protein s1 that promoted complete uncoating. Recently, we identified another variant (named 10M). Variant 10M has a mutation in viral transcriptase cofactor m2 protein which plays multiple roles during virus replication such as formation of viral factories, repression of interferon (IFN) signaling and regulation of apoptosis. T3wt and 10M showed similar levels of s1, host-cell binding and virus uncoating, suggesting that 10M has distinct mechanisms of improved infectivity from our previously described variants T3v1 and T3v2. Per productively infected cells, variant 10M showed up to 5-fold higher levels of m2 and 2-fold higher levels of other viral proteins by western blot analysis, relative to T3wt. Furthermore, 10M produced and released more infectious virions from infected cells at earlier time points. In accordance with enhanced progeny release, 10M induced higher levels of caspase-3 activation. Interestingly, infection by 10M was associated with lower levels of IRF3 phosphorylation, suggesting reduced activation of antiviral signals. We are currently conducting experiments to compare synthesis and stability of T3wt versus 10M m2 transcripts and proteins. We plan to evaluate the impact of m2 mutation on its suggested NTPase and viral factory-formation activities. Whether the mutant m2 has a direct role in modulating cellular responses such as apoptosis and activation of IFN response will be also examined.

Dimas Yusuf

Faculty of Medicine

Department of Medical Oncology

ONCOPRE: a new adjuvant chemotherapy benefit prediction algorithm to assist treatment decision making in colon cancer

Dimas Yusuf, Maria Ho, Winson Cheung

Background: Clinical decision support tools (CDSTs) can help physicians make complex treatment decisions and inform care. For colon cancer, CDSTs such as Adjuvant! Online and Numeracy were widely used to estimate the benefit of chemotherapy and guide conversations with patients. Existing CDSTs, however, do not consider contemporary prognostic factors, such as microsatellite instability (MSI), BRAF mutation status, or the presence of high risk features (HRFs), in their assessment of outcomes. Current CDSTs are also not optimized for modern devices. Methods: We developed ONCOPRE, a chemotherapy benefit calculator for colon cancer that addresses the limitations of existing CDSTs. Based on a review of epidemiological data and results of landmark trials, ONCOPRE was devised to predict 5 year colon cancer recurrence and death. To validate ONCOPRE, we compared its predictions with those generated by existing CDSTs as well as clinical data from tertiary cancer centers across Canada. Results: ONCOPRE can predict 5-year DFS and OS of patients with colon cancer based on age, sex, TNM status, and contemporary risk factors such as MSI status, BRAF mutations, and other HRFs. ONCOPRE's predictions compare favorably with real-world data and predictions from other CDSTs. ONCOPRE's predictions are more optimistic than historical outcomes, and this likely reflects the fact that current day colon cancer patients experience better prognosis with the use of modern therapy and improved supportive care. These attributes make ONCOPRE a potentially new benchmark among CDSTs for colon cancer outcomes. Conclusions: ONCOPRE (<http://www.oncopre.com/>) is a new CDST that can assist in adjuvant treatment decision-making and patient counseling. We make the case that next generation CDSTs in oncology must take into account contemporary clinical, biochemical, and genetic risk factors since these elements significantly affect outcomes. The ONCOPRE platform serves as a potential model on which to develop prediction tools for other forms of cancers.

Alaa Zare

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pediatric

ePIGENETIC AND MOLECULAR DRIVERS OF INFLAMMATION IN
INFLAMMATORY BREAST CANCER

Alaa Zare

Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer that is associated with significant mortality. In spite of advances in IBC diagnoses, the prognosis is still poor compared to non-IBC. It has been estimated that patients diagnosed with Stage IV IBC have less than 6 months survival. Lack of understanding IBC cellular mechanism and the absence of prognostic indicators have contributed to the decrease of survival rate among patients. Due to the aggressive nature of the disease, we hypothesize that inflammation may play an important role in tumorigenesis and metastasis driven by hyperactivation of NF κ B (a transcription factor) as a result of expression loss of RASSF1A (tumor-suppressor protein). Utilizing IBC cell models and patient samples, we were able to determine the epigenetic silencing of RASSF1A in IBC cell lines and patient tissues. The detrimental effects of the expression loss of RASSF1A resulted in hyperactivation of receptor interacting protein kinase 2 (RIPK2). High expression of phosphorylated tyrosine kinase RIPK2 is seen in IBC cell lysate (Western blot) and patient tissues (immunohistochemistry). As a result, elevated production of pro-inflammatory cytokines and growth factors was found in IBC cell culture supernatant suggesting NF κ B upregulation. RIPK2 knock out in IBC cell line showed low cytokine production as a result of NF κ B downregulation. Using several RIPK2 inhibitors identified in our lab, we were able to characterize the downstream effect of inhibiting RIPK2 activity using both proliferation assay and cytokine/chemokine profiling. Inhibition of RIPK2 revealed its ability to regulate tumor proliferation and cytokine production. Our findings suggest that RIPK2 can aid in the diagnosis of IBC patients and would be a novel therapeutic target to restrict NF κ B driven inflammation. Funding Sources: KASP (King Abdullah Scholarship Program)

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Oncology

The RUNX family of transcription factors are oncogenes in granulosa cell tumors of the ovary

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Granulosa cell tumors of the ovary (GCTs), arising from sex cord-stromal cells, accounts for approximately 5% of all ovarian malignancies. Although the prognosis is more favorable than epithelial ovarian cancer, advanced or recurrent tumors show poor outcomes. Treatment options for advanced and recurrent GCTs are still limited. The molecular pathogenesis of GCTs remains poorly understood. The RUNX family of transcription factors (RUNX1-3) are either oncogenes or tumor suppressors in a cancer-specific manner. All three RUNX proteins (RUNX1-3) and CBFbeta (the common heterodimeric partner of RUNX proteins) have been shown to be oncogenes in epithelial ovarian cancer. However, the role for RUNX proteins in GCTs is unknown. We examined the expression of RUNX proteins in SVOG (immortalized granulosa cell line), KGN (adult GCT cell line) and COV434 (juvenile GCT cell line) cells, as well as in human adult GCT tissues by Western blotting. RUNX1 was expressed in all three cell lines, whereas RUNX2 was expressed in KGN and SVOG, but not in COV434. RUNX3 was expressed at a high level in COV434 cells, but was not detected in SVOG and KGN cells. RUNX1 and RUNX2 were expressed in human adult GCT tissues. Functionally, inhibition of RUNX1-3 by a specific inhibitor or by CBFbeta knockdown decreased growth of KGN cells. Overexpression of RUNX3 significantly increased growth, colony formation in soft agar and migration of KGN cells. By contrast, inactivation of RUNX3 by a dominant negative form of RUNX3 reduced growth of COV434 cells. Western blotting and qRT-PCR showed that overexpression of RUNX3 downregulated p27, but increased cyclin D2 expression in KGN cells. Our results suggest that RUNX1-3 could be oncogenes in GCTs. Knockdown experiments are required to further investigate the role for each RUNX protein in GCTs.

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An epigenomic roadmap to PAX3 target gene networks in melanoma

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Melanoma continues to increase in incidence and mortality, accounting for 70% of skin cancer-associated deaths. The developmental origin of melanocytes is thought to be a driver of this aggressive behavior. In this context, melanocyte identity is determined during embryogenesis by the hierarchical action of transcription factors, exemplified by the MITF, SOX10 and PAX3 proteins. Each of these factors has key roles in melanoma, reflecting their capacity to control pathogenic gene expression. Within this scheme, PAX3 acts as a gatekeeper to control cell division and differentiation, yet we do not have a comprehensive view of how it regulates these processes. PAX3 alters gene expression through the recognition of specific target sequences in the genome. To this end, PAX3 contains two DNA-binding domains, the paired domain and the homeodomain. We hypothesize that PAX3 utilizes multiple modes of DNA recognition contributing to melanoma progression through an altered target gene network. We have repurposed data derived from cyclic amplification and selection of targets to model DNA-binding specificities for PAX3 proteins. Significantly, these binding profiles represent the first set of optimal motifs described for full-length PAX3. The robustness of this library was validated in situ by calculating its enrichment across published ChIP-seq datasets for PAX3 and the PAX3-FOXO1 variant. This provided a foundation for predicting PAX3 occupancy across putative cell-specific regulatory regions defined using epigenomic signatures. To identify potential targets of PAX3, we used RNA-sequencing to profile differential gene expression following PAX3 attenuation across melanocyte and melanoma cell lines. Putative targets were subsequently integrated with predicted PAX3 occupancy to connect distinct DNA-binding profiles to transcriptional pathways across cell types. Collectively, these analyses provide novel insight into the discrete target gene networks associated with the differential use of PAX3 DNA-binding modules and how these programs may be altered as melanoma progresses from melanocyte to metastatic disease.

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