10th Annual ICCI

Cancer Research Symposium

Thursday May 25, 2017
OVC LLC 9:00-5:00

Introductory Remarks

Welcome to the 10th Annual Guelph ICCI Cancer Symposium. This meeting is intended to bring together individuals interested in the study of any aspect of cancer in any species, from the most basic elements, to clinical therapies and on to social, emotional and ethical aspects of this often-devastating disease. In addition to campus investigators we welcome local and international researchers. This year in particular we highlight progress in comparative and translational cancer research, and take a look back at some of the research first presented here 10 years ago.

Through interactions facilitated by this meeting, it is hoped that new insights and cooperation will develop that will enhance the research and scholarship in the area of cancer research at the University of Guelph and collaborating institutions. We would like to thank the OVC Dean's Office and the Arthur Willis Visiting Professorship for financial support of the meeting, and for sponsoring the visit of Dr. William Eward, who is this year's Arthur Willis Distinguished Speaker. We hope you will find this symposium interesting and informative, and that it leads to fruitful research partnerships for all our attendees.

Co-Organizers
Brenda Coomber and Tony Mutsaers
Biomedical Sciences and Clinical Studies, University of Guelph

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Thanks to Barb Gaudette, OVC Office of the Dean, for her administrative expertise and invaluable assistance in organizing this event, to David Wood, OVC IT for help with online activities, and to Sharri Norton and her crew at the OVC Dining Hall for help with set up and refreshments. The research projects presented here and the trainees performing these studies were collectively supported by grants, scholarships and contracts from: CIHR; NSERC; Canadian Cancer Society; Cancer Research Society; Terry Fox Research Institute; AAVN-Waltham; Royal Canin; Merial; BioCanRx; Ontario MRI; CFI; OGS; Ontario Trillium Scholarships; Telus Ride for Dad; Prostate cancer Fight Foundation; OVC Pet Trust Fund; Smiling Blue Skies Cancer Fund; Office of the Dean, and Department of Biomedical Sciences, Ontario Veterinary College.

ICCI 10th Annual Cancer Research Symposium, Thursday May 25, 2017

Morning Session: Room 1714, OVC LLC

Moderator: Brenda Coomber

9:00 - 9:05 Welcome and Introductory Remarks: Brenda Coomber

9:05-9:35 *10 Years After: Transgenic Modeling of Breast and Lung Cancer: A 10-Year Journey From Initial Characterization to Mechanistic Insights*
Roger Moorehead; Department of Biomedical Sciences, OVC, University of Guelph

9:35- 9:50 short talk from abstracts
Differential effects of MARRS expression on mammary gland growth and development depend on the Vitamin D3 dose
Allison Wilkin; Department of Human Health & Nutritional Sciences, CBS, University of Guelph

9:50 - 10:15 ***Coffee Break and Poster Viewing*** **Room 1707 B & C, OVC LLC**

10:15- 10:45 *10 Years After: Regulation of cancer cell dynamics by Nck cytoskeletal adaptor proteins*
Nina Jones; Department of Molecular & Cellular Biology, CBS, University of Guelph

10:45 - 11:15 *10 Years After: A decade of anti-angiogenic research in advanced stage ovarian cancer: where we've come and where we're going*
Jim Petrik; Department of Biomedical Sciences, OVC, University of Guelph

11:15- 11:45 *10 Years After: Cross-Species Cancer Comparisons for Driver Gene and Biomarker Discovery*
Geoff Wood; Department of Pathobiology, OVC, University of Guelph

11:45- 12:00 short talk from abstracts
Mitigating the effects of somatic hypermutation on B cell clonality testing in dogs
Mei-hua Hwang; Department of Pathobiology, OVC, University of Guelph

12:00- 1:25 ***Poster Session and Lunch*** **Room 1707 B & C, OVC LLC**

Afternoon Session: Room 1714, OVC LLC

Moderator: Tony Mutsaers

1:30- 2:00 Guest Speaker
Clinical Translation of Gold-nanoparticle-based Radiotherapy for Canine Prostate Cancer Treatment
Shawn Wettig; School of Pharmacy and Waterloo Institute for Nanotechnology, University of Waterloo

2:00- 2:15 short talk
Evaluating the therapeutic potential of oncolytic Newcastle disease virus in mouse models of melanoma and colon carcinoma
Lisa A. Santry; Department of Pathobiology, OVC, University of Guelph

2:15- 2:30 short talk
Combining Oncolytic Viruses with Epigenetic Modifiers in Leukemias
Megan Strachan-Whaley; Department of Pathobiology, OVC, University of Guelph

2:30 - 3:00 *10 Years After: Identifying Patients Who May Benefit from Psychosocial Oncology*
Michèle Preyde; Department of Family Relations & Applied Nutrition, CSAHS, University of Guelph
Pat Chevalier; Social Worker, previously with Supportive Care Department at Grand River Regional Cancer Centre

3:00-3:15 short talk
Sentinel Lymph Node Mapping in Veterinary Medicine
Michelle L Oblak; Department of Clinical Studies, OVC, University of Guelph

3:15 - 3:40 *Snack Break and last chance to view posters* **Room 1707 B & C, OVC LLC**

3:45 - 4:55 Keynote Speaker
Dr. William Eward, Orthopaedic Oncology, Duke Cancer Institute, Duke University, Durham, NC, USA
Sarcomas in dogs and humans: Bringing 'One Medicine' from a hope to a practical reality

4:55 - 5:00 Closing Remarks: Tony & Brenda

KEYNOTE PRESENTATION

3:45 OVC LLC Room 1714

Dr. William Eward, DVM, MD

Assistant Professor of Orthopaedic Surgery, Section of Orthopaedic Oncology
Duke Cancer Institute, Duke University, Durham, NC, USA

Sarcomas in dogs and humans: Bringing 'One Medicine' from a hope to a practical reality

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It was well over 150 years ago with Rudolf Virchow urged the world to see that "between human and animal medicine there is no dividing line – nor should there be." Despite Virchow's foresight, it would be generations before his words were taken seriously. Although sarcomas are seen commonly in dogs, canine sarcomas have been largely ignored by human medical doctors. The price paid is that there has been little progress in the treatment for sarcomas since the 1980s. This talk explores what can be learned by exploring sarcomas across species. Beyond that, we will investigate what the wide and brilliant animal kingdom can teach us about cancer in general? Why do we even have cancer and where in the tree of life is it found? The world of cancer is as vast and strange as the animal kingdom itself. This talk seeks to explore that world a bit deeper. We will review where cancer does and does not thrive within the natural world. We will discuss Peto's paradox – the inscrutable phenomenon whereby large animals should be more susceptible to cancers, and yet they are not. We will discuss some specific comparative oncology projects in the context of One Medicine – and how we are attempting to make the cancer fight an effort across all types of medicine.

Having spent his childhood in the company of a wide variety of animals, Dr. Eward fulfilled a lifelong dream of becoming a small animal veterinarian in 2000 when he graduated from Auburn University's College of Veterinary Medicine. He was particularly captivated by his patients with cancer and decided to pursue this interest further. In 2002, he returned to school, receiving an MD degree from the University of Vermont. He currently is on faculty at Duke University with an adjunct appointment at the North Carolina State College of Veterinary Medicine. He spends the first part of the week taking care of humans with cancer and the latter part of the week taking care of animals with cancer. As an Orthopaedic Oncologist, he specializes in preserving and reconstructing limbs that have been jeopardized by a type of cancer called Sarcoma. Given his dual roles in human and animal health, Dr. Eward is committed to using a One Medicine approach to solving the terrible problem that cancer presents to all of us, whether we walk on two legs or four. He runs a lab at Duke that attempts to identify common elements between types of cancer across different species.

Past ICCI Symposium Arthur Willis Distinguished Speakers

2016	Jaime Modiano	2012	Timothy Fan
2015	Nicola Mason	2011	Cheryl London
2014	Deborah Knapp	2010	Matthew Breen
2013	David Argyle	2009	Barbara Kitchell

GUEST SPEAKERS: PROGRESS & PUZZLES 10 YEARS AFTER

Roger Moorehead
Department of Biomedical Sciences, OVC, University of Guelph
Transgenic Modeling of Breast and Lung Cancer: A 10-Year Journey From Initial Characterization to Mechanistic Insights

The type I insulin-like growth factor receptor (IGF-IR) is a tyrosine kinase receptor that regulates a number of cellular processes including proliferation, apoptosis, migration, differentiation and metabolism. Although the IGF-IR is frequently overexpressed or hyperactivated in a variety of human cancers, its exact role in tumor initiation and/or progression was incompletely understood. To fill this gap in our knowledge, my lab generated transgenic mice that overexpressed the IGF-IR in mammary or lung epithelial cells. At the inaugural ICCI Cancer Research Symposium, I presented the initial characterization of these two transgenic models and demonstrated that overexpression of IGF-IR in the mammary gland or lung was sufficient to induce tumor development, thus highlighting the importance of IGF-IR in tumor initiation. These transgenic mice, and cell lines derived from the tumors, remain integral tools used by my lab. However, our focus has now shifted to the molecular alterations associated with tumorigenesis. The two major projects the lab has been investigating over the last 10 years are (1) understanding the function of a key IGF-IR signaling molecule called, AKT, in lung cancer and (2) investigating how microRNAs (small non-coding RNAs that regulate mRNA translation) regulate mammary tumorigenesis. In addition, we have used molecular profiling of our transgenic models to determine the human cancer subtype we are modelling. These studies are beginning to identify molecules that are essential for tumor cell proliferation and survival which will hopefully lead to novel therapeutic strategies for the treatment of human cancers.

Nina Jones
Department of Molecular & Cellular Biology, CBS, University of Guelph
Regulation of cancer cell dynamics by Nck cytoskeletal adaptor proteins

The adaptor proteins Nck1 and Nck2 are well established signaling nodes in actin cytoskeleton remodeling. Although they were first identified as oncogenes nearly 25 years ago, there is scarce in vivo evidence supporting their ability to induce specific hallmarks of cancer. Our lab has recently demonstrated that in addition to increasing endothelial cell migration and angiogenic remodeling, Nck also promotes endothelial-to-mesenchymal transition (EnMT) during cardiac development, and reports from others indicate a role for Nck in invadopodia formation. These processes are all correlated with invasion and metastasis of breast cancer cells. Accordingly, we have now determined that Nck1 and Nck2 are novel regulators of breast cancer progression, as well as mammary gland morphogenesis. Systemic loss of Nck2 but not Nck1 significantly delays mammary gland development, with decreases in ductal outgrowth and altered terminal end bud formation. Furthermore, using the MMTV-NIC transgenic mouse model of breast cancer, which allows simultaneous expression of activated HER2/ErbB2 and Cre recombinase in mammary epithelial cells, we have shown that deletion of both Nck1 and Nck2 significantly extends

survival and delays tumour onset. Intriguingly, tumours from these animals show evidence of disrupted growth factor signaling, and they are poorly metastatic. As Nck levels are upregulated in aggressive human breast cancers, including HER2+ and triple negative subtypes, our findings suggest that Nck represents an unrecognized molecular determinant of breast cancer.

Jim Petrik
Department of Biomedical Sciences, OVC, University of Guelph
A decade of anti-angiogenic research in advanced stage ovarian cancer: where we've come and where we're going

Our laboratory is interested in the development of novel therapies to treat advanced stage ovarian cancer. Ovarian cancer is typically not detected until late stage where there typically is a large primary tumor on the ovary, metastatic disease in the abdomen, and abdominal distention due to the formation of ascites. Unfortunately, survival from this disease is typically very low due to a lack of effective therapies. Characteristically, the primary and metastatic tumors have abundant vasculature, and the ascites accumulation is due, at least in part, to leaky, fenestrated blood vessels. A decade ago, we hypothesized that an anti-angiogenic approach to inhibit tumor vascularization would induce tumor hypoxia and result in tumor involution and regression. Our anti-angiogenic approach consisted of the use of mimetic peptides against small fragments of a region of the thrombospondin-1 (TSP-1) protein, which is a large endogenous glycoprotein with known anti-angiogenic effects. Well, we were partly right, but mostly wrong. Initial studies showed that we could induce tumor regression with the TSP-1 mimetic peptides and we did induce an anti-angiogenic effect. However, the vascular destruction that we induced appeared to be restricted solely to the abnormal, immature tumor blood vessels, and the healthy, mature, parental blood vessels remained intact. As a result we were left with a small tumor, with normalized vasculature and improved perfusion. After figuring out the implications of our incorrect hypothesis, we felt that we may be able to exploit this vascular normalization and enhanced perfusion to facilitate uptake and delivery of therapeutic compounds such as chemotherapy drugs and oncolytic viruses. In this talk, I will outline our revised approach and present data on our efforts to improve outcomes in advanced stage ovarian cancer with combined vascular normalization and chemotherapy/oncolytic virotherapy

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Geoff Wood
Department of Pathobiology, OVC, University of Guelph
Cross-Species Cancer Comparisons for Driver Gene and Biomarker Discovery

Cancer is fundamentally a genetic disease in that all cancers carry mutations, and genomic instability itself is a hallmark of cancer. Knowing which mutations are functionally important, so-called “driver mutations”, versus those which are non-functional “passenger mutations” is important to our biological understanding of cancer as well as for developing diagnostic tools

and novel therapies. Our approach is to compare spontaneously arising cancers in domestic species to those in humans and leverage both the genetic similarities and differences to find driver mutations. Due to genomic instability in cancers, passenger mutations are relatively randomly scattered across the genome, but our hypothesis is that driver mutations, which cancer cells depend on to sustain their malignant behaviour, will be common across species and stand out among the mutational "noise". To test this idea, we have used array comparative genomic hybridization (aCGH) to find regions of genomic gain and loss in canine osteosarcomas and projected these regions onto published human osteosarcoma aCGH datasets. In a separate study, we are collaborating to conducting whole-exome sequencing of mucosal melanomas in humans, dogs, and horses to find common genetic changes across all 3 species. Many diagnostic and prognostic biomarkers of cancer used in human patients may be of value to companion animals and we are exploring these using high-throughput immunohistochemistry of canine tumours and PCR arrays to measure circulating microRNA in dogs with cancer. The overall vision of these investigations is to take advantage of the common biology of cancer across species towards better diagnostics and therapeutics in both humans and animals.

Michèle Preyde
Department of Family Relations & Applied Nutrition, CSAHS, University of Guelph
Craig Cunningham
Grand River Regional Cancer Centre (GRRCC), Kitchener, ON
Pat Chevalier
Social Worker, previously with Supportive Care Department at GRRCC
Identifying Patients Who May Benefit from Psychosocial Oncology

The primary focus of this collaborative research program concerns the identification of the psychosocial needs of patients with cancer who are accessing a regional cancer centre (GRRCC). At GRRCC, some psychosocial supports are available, though it is not known if these supports are fully meeting the needs of this patient group. In our previous research at GRRCC including all cancer patients (Preyde et al., 2010) and patients with prostate cancer (Preyde et al., 2012), two main areas were identified as critical: 1. a high volume of patients indicating anxiety and other psychosocial experiences that are separate from depression on a self-administered screening tool; and 2. the distress associated with not knowing one's personal outcomes. The current project (still under REB review) is focussed on patients with prostate cancer and will include a sexual health needs assessment which has been identified by patients and staff alike as needing attention. The hospital has adopted a Cancer Care Ontario tool to identify physical symptoms (e.g., urinary function) which contains 2 psychosocial items (feeling depressed and lack of energy) on a 4-point scale (1-4). Those who score 3-4 on feeling depressed will be referred automatically to social work services. For other psychosocial concerns such as anxiety and sexual health struggles, the social work services rely on the clinical team to assess and make referrals. Anxiety is the most common psychiatric symptom of patients with prostate cancer (Roth et al., 2008). Identifying people who may benefit from supportive care is a complex process situated in a complex system of health care delivery. The purpose for this presentation is

to shed light on some of the psychosocial experiences of cancer, and some of the difficulties in aligning need to support.

Roth, AJ, Weinberger, MI, Nelson, CJ. Prostate Cancer: Quality of Life, Psychosocial Implications and Treatment Choices. *Future Oncol.* 2008 Aug; **4**(4): 561–568

Preyde M, Hatton-Bauer J, Chevalier P, Barksey M. Exploratory Survey of Patients' Needs and Perceptions of Psychosocial Oncology. *J. Psychosocial Oncol.* 2010, **28**(3), 320-333.

Preyde, M., Hatton-Bauer, J., Cunningham, C., Panjwani, D., Evaluation of an informational pamphlet on distress and perceptions of supportive care for men with prostate cancer. *J. Men's Health*, 2012, **9**(3), 160-67

INVITED LOCAL SPEAKER

Shawn Wettig
School of Pharmacy and Waterloo Institute for Nanotechnology, University of Waterloo

Clinical Translation of Gold-nanoparticle-based Radiotherapy for Canine Prostate Cancer Treatment

Prostate cancer is the third leading cause of death from cancer in men in Canada. Typical external beam radiotherapy of prostate cancer consists of 20-39 radiation treatments over an 8-week period. Alternatively, brachytherapy has fewer hospital visits but is highly invasive with multiple side-effects and long recovery-time from tissue damage. In place of these radiotherapy options, our research envisions a single injection of radioactive/non-radioactive gold nanoparticles (functionalized with nutraceutical), delivered using minimally-invasive prostatic artery embolization (PAE) to canine patients. Although PAE has been successfully used for the treatment of early stage prostatic diseases, and the radio-sensitization, dose enhancement and imaging effects of gold nanomaterials have also been previously reported, the unique combination of these modalities has not yet been investigated. To investigate the therapeutic efficacy of PAE along with functionalized gold nanoparticles in larger animals like dogs, it is critical to evaluate the biodistribution, preferential uptake and retention within tumour, toxicity to vital organs, and dose enhancement of functionalized gold nanoparticles in smaller animals such as the mice model. This research has the potential to provide an alternative treatment option with a single injection followed by as few as just one external beam radiation treatment. Nanoparticle therapy (with PAE in dogs) offers a significant improvement in the quality of life for cancer patients by reducing the treatment burden while at the same time promising higher cure and lower complication rates.

SHORT TALKS FROM SUBMITTED ABSTRACTS
(*Study Leader)

Morning Session

Differential effects of MARRS expression on mammary gland growth and development depend on the Vitamin D3 dose
Allison Wilkin, Robert Sullivan, Thao Trinh, Michael Edson, Kelly Meckling*
Department of Human Health & Nutritional Sciences, CBS, University of Guelph

1,25 dihydroxyvitamin D3 (1,25D3) is one of the most potent biologically active forms of vitamin D3. 1,25D3's effects include cell growth inhibition, induction of differentiation, and anti-cancer effects, depending on the target tissue. Most of the activities of 1,25D3 continue to be ascribed to the classic vitamin D receptor despite the identification of another membrane receptor: MARRS. The purpose of this study was to explore the role of MARRS in the mammary gland using a conditional knockout mouse model and a vitamin D3 dietary intervention. MARRS was reduced in epithelial cells of mammary glands (MG) using the Cre/loxp system. 4th MGs were collected from 6-week old female MMTV-Cre mice (n=94) on diets of 10,000 IU/kg, 1,000 IU/kg, or 0 IU/kg of D3. Growth of MG's was measured by counting the number of terminal end buds (TEB) of alveolar branches, the length of the longest ductal extension, and total MG area covered by ducts. There was a significant interaction between genotype and diet regarding TEBs ($p=0.001$) and ductal coverage ($p=0.026$). Knockout mice on the 1,000IU/kg diet had significantly fewer TEBs ($p=0.001$) compared to wildtypes on the same diet, but the opposite effect was seen in mice on the 10,000IU/kg diet. These results suggest that there is an effect of MARRS on mammary gland development that is dependent on vitamin D status. Since the mammary gland during puberty is highly proliferative, it has been proposed as a target for carcinogens, and therefore altered growth may affect breast cancer risk later in life.

Funding: NSERC

Mitigating the effects of somatic hypermutation on B cell clonality testing in dogs
Mei-hua Hwang[1], Nikos Darzentas[2], Dorothee Bienzle[1], Peter Moore[3], Stefan Keller[1]*
[1]Department of Pathobiology, OVC, University of Guelph; [2]CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [3]Pathology, Microbiology & Immunology, University of California, Davis, CA, USA

Clonality testing is a molecular assay to diagnose lymphoma if traditional methods such as cytology or histopathology are equivocal. Clonality testing assesses the clonal diversity of lymphocytes in a given sample by PCR-based amplification and size separation of a hypervariable lymphocyte antigen receptor gene. The sensitivity of B cell clonality assays hinges on two main factors: the degree of coverage of germline immunoglobulin heavy chain (IGH) sequences by primers of the assay, and how well the assay accounts for primer site aberrations introduced by somatic hypermutation (SHM). Despite constant improvements over the last

decade, clonality assays still suffer from a high false-negative rate, i.e. cases of lymphoma go undetected. This is in part because recent studies have focused on improving the primer coverage but have insufficiently addressed the effect of somatic hypermutation on the sensitivity of clonality assays. The objective of this study was to assess the patterns and magnitude of SHM at the canine IGH locus as a basis for an improved clonality assay that accounts for and mitigates the negative effects of SHM. Somatic hypermutation was investigated by high-throughput sequencing of canine IGH transcripts generated by 5'RACE amplification of cDNA. These results will facilitate the designing of a novel, multiplexed primer set with improved sensitivity by accounting for recurring patterns of SHM within putative primer binding sites. The novel assay is expected to improve the timeliness of diagnosis, and enhance the quality of care for patients of canine lymphoproliferative disease.

Funding: NSERC, OVC Pet Trust Fund, OVC Scholarship

Afternoon Session

Evaluating the therapeutic potential of oncolytic Newcastle disease virus in mouse models of melanoma and colon carcinoma

Lisa A. Santry[1], Amanda Au Yeung[1], Thomas M. McAusland[1], Jacob P. van Vloten[1], Rob C. Mould[1], Kathy Matuszewska[2], Byram W. Bridle[1], James J. Petrik[2], Sarah K. Wootton[1]*
[1]Department of Pathobiology, [2]Department of Biomedical Sciences, OVC, University of Guelph

Newcastle disease virus (NDV) is a negative ssRNA virus in the Paramyxoviridae family. NDV selectively replicates in tumour cells due to defects in antiviral and apoptotic signalling. In addition to its direct oncolytic effect, NDV also activates both innate and adaptive immune responses. While NDV has demonstrated efficacy in various cancer models, little is known about the mechanism involved in the increased survival and reduction in tumour growth. To this end, we evaluated the oncolytic potential of rNDV-GFP in murine melanoma and colon carcinoma cell lines using a resazurin cell viability assay. Infection with rNDV-GFP at a range of multiplicity of infection (MOI) significantly reduced the viability of these cells in vitro. Furthermore, we evaluated the ability of these cells to support the replication of NDV and found only limited virus. These results suggest that a low level (if any) replication is required for NDV to evoke its oncolytic potential. In vivo experiments utilizing intradermal melanoma (B16-F10) and subcutaneous colon carcinoma (CT26-LacZ) models revealed that administration of NDV via intravenous and intratumoural delivery lead to early shutdown of NDV replication and transgene expression in tumour cells with intact type IFN signalling. Furthermore, flow cytometry analysis of immune cell profiles demonstrate that NDV is able to activate immune cells in both the lymph nodes and within the tumour. Together these results suggest that NDV is a strong candidate for immunotherapy and may indicate that replication and subsequent transgene expression is limited in tumour cells with intact type I IFN signalling pathways.

Funding: NSERC

Combining Oncolytic Viruses with Epigenetic Modifiers in Leukemias
Megan Strachan-Whaley, Lisa Santry, Amanda Au Yeung, Byram Bridle*
Department of Pathobiology, OVC, University of Guelph

Acute leukemias are blood cell cancers categorized as lymphoid or myeloid. While chemotherapies for B –cell leukemia achieve a survival rate of approximately 90%, the prognosis for T-cell (T-ALL) and acute myeloid leukemias (AML) remains poor. One alternative to chemotherapy is oncolytic viruses (OVs) including vesticular stomatitis virus (VSV), maraba virus (MG1), and Newcastle disease virus (NDV). OVs fail in leukemia, possibly due to clearance of the virus by host immunity. Epigenetic modifiers (EMs) including histone deacetylase inhibitors like SAHA and MS275, and DNA methyltransferase inhibitors like decitabine also have efficacy in leukemias but are unable to control disease. We hypothesize that cells resistant to OVs may be particularly sensitive EMs. Using a T-ALL model, combination therapy produces significantly more cell death than monotherapies in-vitro. Cell death and proliferation assays demonstrate that OVs have primarily cytotoxic effects, while EMs have primarily cytostatic effects. Cells treated with OV followed by EM showed both cytostatic and cytotoxic effects. In-vivo studies strongly support our hypothesis, with mice treated with NDV followed by DCB surviving significantly longer than controls. Furthermore, mice treated with MG1 followed by DCB all achieve durable cures. Therapeutic efficacy of EMs is believed to be limited by upregulation of drug pumps by tumour cells. Ongoing experiments explore the hypothesis that exposure to OVs leads to down-regulation of these pumps by tumour cells, making them more vulnerable to EM therapy.

Funding: Canadian Cancer Society, Terry Fox Research Institute, OVC Scholarship

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Sentinel Lymph Node Mapping in Veterinary Medicine
ML Oblak*, A ZurLinden, S Nykamp
Department of Clinical Studies, OVC, University of Guelph

Identification of metastatic disease is critical for determining the extent of disease, prognosis, and for developing treatment plans. For many cancer types, metastasis occurs via the lymphatic system and the status of the draining lymph nodes is an important part of the pre- and intraoperative evaluation. Sentinel lymph node (SLN) techniques rely on the fact that typically 1-2 lymph nodes are the primary means of drainage of a tumour and these nodes will act as a marker for the status of the rest of the nodal bed. With SLN sampling, in contrast to nodal bed excision, only the SLNs are removed, resulting in decreased morbidity of the procedure. In veterinary patients, fewer LN exist but removal can still be associated with complications, especially in the head and neck region. Currently there is minimal literature and no developed technique for SLN evaluation in veterinary patients. In addition, imaging criteria of metastasis are not well established and therefore multiple LN are routinely dissected and submitted for histopathology, even if they appear normal on preoperative staging. The ability to better identify the most relevant lymph nodes to remove would decrease the effect on the patient while not compromising treatment. This pilot study describes a technique for identification of SLN in

veterinary clinical patients with oral tumours. This technique combines the use of preoperative CT lymphography with intraoperative peritumoral blue dye injection to identify suspected sentinel lymph nodes with histopathology of all lymph nodes used as the gold standard for comparison.

Funding: OVC Pet Trust Fund

LUNCH & POSTER SESSION 12:00-1:25

OVC LLC Room 1707 B & C

Posters will be displayed all day; authors please attend your poster 12:30-1:00

POSTER ABSTRACTS

(*Study Leader)

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1) *In vitro evaluation of a positive pressure CO_2 environment on canine TCC*

Rashi Asthana, Michelle Oblak*, Ameet Singh, Anthony J. Mutsaers
Department of Clinical Studies, OVC, University of Guelph

Introduction: Laparoscopy is commonly being performed in canine patients with cancer and the potential effects of CO_2 insufflation on growth and dissemination of neoplastic cells is unknown. The purpose of this study was to determine cell viability, proliferation, and migration of canine transitional cell carcinoma (TCC) following exposure to in vitro positive pressure CO_2 environment. Methods: Canine TCC and MDCK cells were exposed to 100% CO_2 (21°C) at 0, 5, 10 and 15 mmHg for 2 hours using an insufflator at 37°C. Culture media pH was measured. Viability, proliferation, and migration were assessed using Trypan Blue Exclusion Dye, Resazurin Reduction assay, and Scratch Migration assays, respectively. Three-way ANOVA and One-way ANOVA were used for statistical analyses with a $p<0.05$ considered significant. Results: pH was significantly decreased immediately after CO_2 exposure ($p<0.001$) but returned to normal 1-hour post exposure. Viability was significantly ($p<0.001$) affected by different pressures of CO_2 insufflation in both TCC and MDCK cells. Although the trend is not linear, 5 and 10 mmHg had a more significant effect than 15 mmHg when compared to control and 0 mmHg in both TCC and MDCK cells. Pressure had no significant effects on proliferation ($p = 0.9472$) and migration ($p = 0.97$) in any of the experiments. Conclusion: A positive pressure CO_2 environment causes a significant change in cell viability of TCC and MDCK cells without affecting their proliferation or migration. Due to the varied physiological effects of the abdominal microenvironment, this work warrants further in vitro and in vivo study.

Funding: OVC Scholarship

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2) *Acute virus-induced leukopenia: challenging the cell trafficking paradigm during oncolytic virotherapy.*

Amanda WK. AuYeung[1], Robert C. Mould[1], Jacob van Vloten[1], Mahi Azizi[1], J. Paul Woods[2], Geoffrey Wood[1], James J. Petrik[3], Khalil Karimi[1], Byram W. Bridle[1]*;
[1]Department of Pathobiology, [2] Department of Clinical Studies, and [3] Department of Biomedical Sciences, OVC, University of Guelph, Guelph, Ontario, N1G 2W1

The current paradigm is that oncolytic viruses (OVs) induce pro-inflammatory cytokines and chemokines that recruit and activate tumour-infiltrating leukocytes (TILs). However, we hypothesize that OV-induced leukopenia acutely decreases TILs. We quantified TILs in

intradermal B16-F10 melanomas, blood and spleens of mice treated intravenously with 1e9 pfu of vesicular stomatitis virus (VSV; Δm51 mutant). The number of TILs significantly decreased 24 hours post-infection (hpi). There was a profound loss of B cells and reduction of macrophages, myeloid-derived suppressor cells (MDSCs), and CD4+ T cells, except those with a regulatory phenotype (Tregs). Only neutrophils were more numerous post-treatment. Total TILs returned to baseline by 72 hpi, at which time CD8+ T cells and Tregs had increased above baseline. There were more intratumoural NK cells at 72 hpi but they were no longer activated. Macrophages and B cells remained at low numbers in tumours at 72 hpi. We extended our study to include Newcastle disease virus and Orf virus. Both OVs induced profound leukopenia in blood at 24 hpi but the total number of TILs was unchanged. Only MDSCs decreased among TILs, while both viruses substantially increased intratumoural NK cells and neutrophils. In contrast to the current paradigm, we show that many leukocytes leave tumours following treatment with VSV. For other OVs, intratumoural reduction of leukocytes is not apparent. However, all OVs acutely altered the presence of various leukocyte subsets. Understanding how OVs modulate trafficking of TILs can provide strategies to alter the intratumoural cytokine/chemokine milieu to optimize immunological cell profiles.

Funding: National Centre of Excellence in Biotherapeutics for Cancer Treatment (BioCanRx), OVC Pet Trust Fund, OVC Pet Trust Scholar Program, Canadian Graduate Scholarship-Master's (CIHR)

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3) *Targeting mTOR for Chemotherapy Sensitization in Canine Osteosarcoma Cells*

Chesney A. Baldwin[1], Amy L. Westlund[1], Jodi Morrison[1], Anthony J. Mutsaers[1,2]*
[1]Department of Biomedical Sciences, [2]Department of Clinical Studies, OVC, University of Guelph.

Osteosarcoma (OSA) is the most common primary bone tumour in canines. Despite aggressive treatment with amputation followed by systemic chemotherapy, the mean survival time is less than one year after diagnosis due acquired drug resistance to chemotherapeutics. There has been little advancement in survival time within the last 30 years and therefore, it is essential to explore alternative treatment strategies. The PI3K/mTOR pathway has emerged as a novel target in OSA treatment as it is commonly over-activated resulting in increased cellular proliferation, survival, metabolism, and chemoresistance. Rapamycin, a small molecule inhibitor of mTOR is currently undergoing clinical evaluation in canine OSA. In addition to carboplatin, doxorubicin is another standard-of-care chemotherapeutic agent for OSA treatment. The effects of mTOR inhibitors as well as doxorubicin and carboplatin chemotherapy, on mTOR pathway modulation, viability, and metabolism were assessed in vitro on 4 canine OSA cell lines. Combination treatment resulted in a synergistic reduction of cellular viability, measured by a resazurin reduction assay. Combination treatment also prevented chemotherapy-induced pathway activation of downstream components 4EBP1 and p70S6K determined by western blotting. However, surprisingly there was no effect on mTOR activation following treatment. In addition, combination treatment decreased glycolysis, a key component of cancer metabolism, when compared to chemotherapy only treatment. Results showed that simultaneous mTOR inhibition with chemotherapy treatment lead to greater anti-cancer effects than the single agent chemotherapy treatment. Inhibiting

mTOR may have the potential to be translated into clinical practice to improve canine OSA treatment with either doxorubicin or carboplatin chemotherapy.

Funding: OVC Pet Trust Fund

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4) *Comparative evaluation of EGFR and HSP family proteins and cancer stem cell genes in primary and metastatic cell lines isolated from genetically engineered mouse models of osteosarcoma*

Steven Baltjes, Katie Landon, Brooke Fraser, Anthony Mutsaers*
Department of Biomedical Sciences, OVC, University of Guelph

Osteosarcoma is the most common primary bone tumor in humans and dogs. While the tumor may be removed successfully, development of metastatic disease limits survival. Unfortunately, treatment decisions aimed at metastatic disease are based on information from the primary tumor. Transgenic mouse models of osteosarcoma that develop metastasis provide an opportunity to compare potential treatment targets between primary and metastatic lesions that develop in these mice. We compared expression of EGFR and HSP family proteins in cell lines established from paired primary and metastatic lesions. Additionally, as osteosarcoma conforms to a cancer stem cell model, we also compared sarcosphere formation and gene expression of stem cell markers Nanog, Oct4, STAT3, Sox2, and CD133 in these cell lines. Finally, to assess comparative differentiation of cells in both monolayer and sarcosphere conditions between primary and metastatic cell lines, gene expression of DMP-1 and osteocalcin was quantified. EGFR, HER2, HSP70 and GRP78 were increased in metastatic compared to primary cell lines. Significant differences in sphere forming ability were observed, but sphere formation was not consistently higher in the metastatic cell lines. Sarcospheres had increased expression of Nanog, Oct4, CD133 and Sox2 compared to adherent cultures. Primary tumor cells had higher expression of DMP-1 than metastatic cell lines, and sarcospheres displayed increased DMP-1 and osteocalcin compared to the same cells grown in monolayer. Ongoing work is validating these results in a larger sample size, to confirm the possibility that potentially treatable targets may have higher expression in metastases compared to the primary tumor.

Funding: Office of the Dean, OVC; Department of Biomedical Sciences

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5) *Mutation of Leucine-8 to Lysine in Syntaxin4 Impairs Binding to Munc18c, Invadopodium Formation and Gelatin Degradation*

Megan Brasher, Andrea Hucik, Marc Coppolino*
Department of Molecular & Cellular Biology, CBS, University of Guelph

Tumor cell invasion involves the localization of proteins required for ECM (extracellular matrix) interaction and proteolysis. The targeted localization of many components during cell-ECM interactions is dependent on membrane trafficking, mediated in part by soluble N-

ethylmaleimide-sensitive factor (NSF) attachment protein receptors (SNAREs). Recent studies indicate that the SNARE Syntaxin4 (Stx4) is involved in the formation of invadopodia (specialized degradative structures formed during tumor cell invasion); however, it is not clear how Stx4 function is regulated during tumor cell invasion. Munc18c is a known regulator of Stx4 activity, and here we show that Leucine 8 of Stx4 is required for this interaction. Biochemical and microscopic analyses revealed that mutated Stx4 is unable to interact with Munc18c. MDA-MB-231 cells overexpressing mutated Stx4 were analyzed for their ability to form invadopodia and degrade gelatin in vitro. These cells were found to have decreased invadopodia formation and gelatin degradation, compared to cells overexpressing wildtype Stx4. These findings suggest that Munc18c regulates Stx4 through Leucine 8, and this interaction is required for cell invasion.

Funding: NSERC

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6) *Low oxygen influences the composition of the human ribosome and the regulation of ribosomal protein genes*

Andrea Brumwell, Lorian Fay, Jim Uniacke*
Department of Molecular & Cellular Biology, CBS, University of Guelph

Ribosomes have long been considered to be tightly regulated in their composition because of their essential role as molecular machines responsible for catalyzing protein synthesis. They possess a high degree of kingdom-wide conservation, and many mutations in ribosomal components are lethal. This view is changing, as mutations in certain ribosomal proteins are tolerated by cells, albeit with disease phenotypes known as “ribosomopathies”. Additionally, specialized ribosomes have been observed in stressed bacteria and yeast cells that show transcript specificity during translation. Here, we show that ribosomal protein complement of the human ribosome is influenced by low oxygen (hypoxia), a key feature of the tumor microenvironment. We quantified ribosomal protein levels in actively translating ribosomes by Tandem Mass Tags mass spectrometry. Our data suggest that human ribosomes are heterogeneous, and that select ribosomal proteins are more likely to be incorporated into hypoxic ribosomes than their normoxic counterparts. Furthermore, hypoxia affected the expression of over a third of ribosomal protein genes and induced five alternative splicing events within a subset of these genes. We demonstrate that these five alternative splicing events are signatures of tumor hypoxia in cancer cells and human tumors. This study provides insight into mechanisms of hypoxic gene expression that are active within tumor cells.

Funding: NSERC, OGS

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7) *Inhibition of AKT-1 for the Treatment of Human Non-Small Cell Lung Cancer (NSCLC)*
Paige Chorner, Roger A. Moorehead*
Department of Biomedical Sciences, OVC, University of Guelph

AKT is a serine-threonine kinase implicated in tumorigenesis as a central regulator of cellular growth, proliferation, survival, and metabolism. Activated AKT is overexpressed in 50-70% of NSCLC tumors and has exhibited an association with poor prognosis as well as chemotherapeutic resistance to platinum-based therapy. Accordingly, AKT inhibitors such as MK-2206 are currently undergoing clinical investigation for the treatment of human NSCLC however, these agents broadly target all three (1-3) AKT isoforms. Recent evidence suggests opposing roles of the AKT isoforms in tumorigenesis where loss of AKT-1 inhibits while the loss of AKT-2 enhances lung tumor development in a transgenic mouse model. Based on these findings, we hypothesize that preferential inhibition of AKT-1 would warrant a more effective therapeutic strategy for NSCLC compared to the current clinical approach of broad AKT inhibition. The AKT-1 inhibitor A-674563 has proven to be a more potent regulator of NSCLC cell survival compared to the pan-AKT inhibitor MK-2206. Neither inhibitor stimulates apoptosis at their determined IC50 values but they induce divergent effects on cell cycle progression. In addition, off-target CDK2 inhibition likely plays a vital role in the improved efficacy of the AKT-1 inhibitor A-674563. Similar reductions in cell viability were observed with the CDK2 inhibitor PHA-848125. In addition, cell lines with higher CDK2 and p-CDK2 expression are marginally more sensitive to the AKT-1 inhibitor A-674563 compared to the pan-AKT inhibitor MK-2206.

Funding: Canadian Cancer Society, OGS

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8) *Sex disparity in innate immune responses to recombinant vesicular stomatitis virus: the role of type I interferon signaling and neutrophils*
Maedeh Darzianiazizi, Katrina E. Allison, Khalil Karimi*, Byram W. Bridle*
Department of Pathobiology, OVC, University of Guelph

Anti-tumour functions of oncolytic viruses (OVs) rely on host immune stimulation. However, host responses against OVs also limit their replication. Innate antiviral responses are initiated and largely controlled by type I IFNs signaling through interferon-α/β receptors (IFNAR). Moreover, neutrophils are the first innate cells recruited to sites of infection and can exhibit both protective and pathologic functions. We investigated the role of IFNAR-mediated signaling on cytokine responses and neutrophil trafficking to blood in female and male mice infected with an attenuated recombinant vesicular stomatitis virus (rVSVΔm51). Pre-treatment with an IFNAR-blocking antibody produced higher levels of the pro-inflammatory cytokines IL-6, IL-12, KC and TNF-α in response to OV infection. Anti-IFNAR-treated males had exaggerated cytokine expression compared to males with intact IFNAR signaling at 10hrs post-infection (hpi), followed by reduction of cytokines to normal levels at 24hpi. However, females pre-treated with anti-IFNAR experienced even more exaggerated cytokine responses than female controls and all males at 10hpi. These responses remained elevated at 24hpi, suggesting that females have a

reduced ability to negatively regulate cytokines during IFNAR blockade. Additionally, during IFNAR blockade, there was a huge increase in the number of neutrophils in the blood of males at 24hpi. Interestingly, depletion of neutrophils in both male and female animals led to huge elevations in cytokines after rVSVΔm51 infection. Our observations indicate a regulatory role of IFNAR-mediated signaling in cytokine responses that appears to be affected by sex. Furthermore, the results suggest a regulatory role for neutrophils in antiviral cytokine responses, irrespective of sex.

Funding: NSERC, Ontario Trillium Scholarship

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9) *Construction and validation of a novel vaccine for the treatment of canine oral melanomas*

Li Deng, Robert C. Mould, Julia Kim, Wing Ka Amanda AuYeung, Byram W. Bridle*
Department of Pathobiology, OVC, University of Guelph

Oral melanomas in dogs (a translational model for human melanomas), are aggressive, metastatic and associated with survival rates of only 6, 3 and 1 month(s) for stage II, III and IV tumours, respectively, even with surgical excision. In collaboration with the Toronto Recombinant Antibody Centre, we aim to test synthetic canine immunomodulatory antibodies in combination with a cancer vaccine to treat canine oral melanomas. Therefore, we designed a new vaccine with the objective of inducing robust canine melanoma-specific T cell responses amenable to quantitative and qualitative assessments by flow cytometry to facilitate evaluation of the immunomodulatory potential of the synthetic antibodies. Our vaccine utilizes an E1/E3-deleted recombinant human serotype 48 adenovirus (rHuAd48) expressing canine melanoma antigen recognized by T cells-1 (cMART1) and human dopachrome tautomerase (hDCT) to provide homologous and heterologous T cell targets, respectively. cMART1 was selected because it was highly overexpressed in canine melanomas relative to matched normal skin, as assessed by western blotting. The vaccine is designed to induce high-affinity CD4+ helper T cells to support cMART1- and cross-reactive canine DCT-specific CD8+ cytotoxic T cells. Identity testing by western blotting demonstrated proper construction of the vaccine. We confirmed that rHuAd48 expressing enhanced green fluorescent protein could infect canine cells and mediate transgene expression. Further, we have optimized a flow cytometry assay for assessing canine T cell responses following stimulation with PMA and ionomycin. We are conducting pre-clinical safety testing and mapping T cell epitopes in mice.

Funding: OVC Pet Trust Fund, National Centre of Excellence in Biotherapies for Cancer Treatment (BioCanRx)

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10) *Canine T-cell lymphoma: cytomorphological, histomorphological, immunohistochemical, and flow cytometric analysis*

Nariman Deravi[1], Olaf Berke[2], Anthony Mutsaers[3], Michelle Oblak[3], Stefan Keller[1], Veronica Parsons[1], Dorothee Bienzle[1]*

[1]Department of Pathobiology, [2] Department of Population Medicine, and [3] Department of Clinical Studies, OVC, University of Guelph

Background: Lymphoma common in dogs frequently treated with chemotherapy and grouped into B- or T-cell types using CD79a or CD21 (B) and CD3 (T) markers. T-cell lymphoma is considered to have a worse prognosis than B-cell lymphoma, however, there are different types of T-cell lymphoma with variable biological behaviour. Few studies have examined whether prognosis differs between subtypes of T-cell lymphoma. Objective: The objective of this study was to determine prognosis of patients with T-cell lymphoma characterized by cytopathology, histopathology, immunohistochemistry, and flow cytometry. Methods: Lymph node aspirates/biopsies were collected from dogs with T-cell lymphoma. Flow cytometric analysis included detection of CD3, CD4, CD8, CD21, CD22, CD45, and MHCII antigens. Formalin-fixed paraffin embedded sections were assessed for architecture and cytomorphology, and by immunohistochemistry for expression and location of CD3, CD79a, and granzyme B. On frozen sections, CD4, CD8, and CD21 expression was determined. Tumors were classified following World Health Organization guidelines, and results compared to flow cytometry and cytopathology results, response to chemotherapy and survival. Results: Flow cytometrically, four cases were CD8+, three were CD4+, and four were CD4-/CD8 . Histomorphologically, tumors had diffuse architecture with peripheral displacement and compression of remnant follicles. Tumors diffusely expressed CD3; four were granzyme positive, and CD79a expression was restricted to follicles. In the frozen sections, CD4, CD8, and CD21 were expressed diffusely when present with varying levels of intensity. Conclusions: Based on preliminary analysis, patients with CD8+ T-cell lymphoma had a different response to chemotherapy than those with CD4+ lymphoma.

Funding: OVC Pet Trust Fund

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11) *Apigenin Regulates the Expression of Cell-Surface CD26 but Not Total CD26 Protein on Colon Epithelial Cells*

Bogdan Diaconu[1], Emilie C. Lefort[2], Jonathan Blay[1,2]*

[1]School of Pharmacy, University of Waterloo; [2]Department of Pathology, Dalhousie University

Apigenin is a plant flavonoid found primarily in parsley and chamomile products. It is typically found in the diet as a glycoside which is then cleaved by microbial and brush border enzymes in the intestine. This process releases apigenin which is absorbed by cells of the epithelium. We used established cell lines of HT29 human colorectal epithelial cells to investigate how apigenin influences the display of the CD26 enzyme. CD26 is a multifunctional cell-surface receptor. It binds adenosine deaminase which in turn degrades extracellular adenosine. CD26 also has

peptidase activity that cleaves the chemotactic compound CXCL12, and it can bind directly to the extracellular matrix proteins fibronectin and collagen. We have shown using a radio-antibody binding assay that cell-surface CD26 expression increases following apigenin treatment. The greatest increase in surface CD26 expression occurs after a 24h treatment with 100μM apigenin. However, western blotting shows that total cellular CD26 protein does not change significantly following 24h apigenin treatment. This suggests that apigenin may affect the intracellular transport of CD26 from the cytoplasm to the cell surface.

Funding: NSERC

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12) *Characterizing Changes in Dipeptidyl Peptidase IV on Colo Cancer Cells in Response to Prostaglandin Treatment*

Alexandra Durocher, Jonathan Blay*
School of Pharmacy, University of Waterloo

Dipeptidyl peptidase IV (DPPIV) is a multifunctional transmembrane protease that is found on most human cells, and shows variable expression between different types of cancer. DPPIV activity may inhibit cancer progression by interacting with several components of the tumour microenvironment (TME). DPPIV also degrades several signaling molecules in the TME including the chemokine CXCL12. CXCL12 has shown to promote tumour progression when binding to its specific cell-surface receptor CXCR4. CXCR4 expression on colon cancer cells decreases after treatment with j-series prostaglandins, which are endogenous fatty acid derivatives that have shown to confer anti-metastatic properties on cancer cells. Prostaglandin binding to the peroxisome proliferator receptor gamma (PPARγ) decreases CXCR4 expression. Downregulated CXCR4 may be accompanied by upregulated levels of DPPIV, since CXCR4 and DPPIV generally exhibit an inverse relationship. We are investigating whether prostaglandin treatment indirectly increases cell-surface DPPIV expression and activity on colon cancer cells by activating PPARγ. Our results thus far have shown an increase in DPPIV activity after treatment with prostaglandins 15d-PGJ2 and PGD2, but this effect was not seen with PGJ2 and PGF2α. Furthermore, prostaglandin treatment has not shown to affect DPPIV mRNA levels. We plan to examine the potential impact of prostaglandins on metastasis by observing their effects on CXCL12-mediated cell migration. Upregulating DPPIV may allow us to exploit its anti-metastatic properties.

Funding: NSERC

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13) *DEAD-box polypeptide DDX28 is a negative regulator of cap-dependent translation under low oxygen conditions*

Sonia L. Evagelou, James Uniacke*
Department of Molecular & Cellular Biology, CBS, University of Guelph

The most common form of translation initiation in eukaryotes occurs in a cap-dependent manner, which requires the recruitment of the eukaryotic translation initiation factor 4F (eIF4F) to the m7GTP cap located at the 5' end of all cellular mRNAs. eIF4F is a heterotrimeric protein complex composed of the cap binding factor eIF4E, the RNA helicase eIF4A, and the scaffolding protein eIF4G, which together function to initiate cap-dependent translation. However, in response to hypoxia, a common feature of the tumour microenvironment, intricate signaling pathways are activated that culminate in the inhibition of eIF4E. Recently, it was discovered that hypoxic cells are able to utilize an alternate 5' cap binding mechanism, whereby cells switch to the use of the eIF4E homologue, eIF4E2, in order to maintain selective cap-dependent translation of critical hypoxic mRNAs. While there is some understanding of how this hypoxic translation initiation complex, eIF4FH, is functioning, there is still little known about how it is regulated. We hypothesize that DDX28, a DEAD-box RNA helicase family member, acts as a negative regulator of eIF4E2-mediated translation. Our data from polysome profile analysis, co-immunoprecipitation, and cap-affinity assays suggest that a decrease in DDX28 protein expression under hypoxia may serve to enhance the cap-binding affinity and overall activity of eIF4E2. Given the novelty of this hypoxic cap-dependent translation mechanism, identification and characterization of eIF4FH regulators will not only aid in our understanding of translational versatility, but could also ultimately lead to the development of cancer-therapeutics that selectively target the hypoxic protein synthesis machinery.

Funding: NSERC, OGS

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14) *β_1 Integrin-Mediated Regulation of MT1-MMP Phosphorylation in Invasive Tumour Cells*

Olivia Grafinger, Marc G. Coppolino*
Department of Molecular & Cellular Biology, CBS, University of Guelph

The majority of all cancer-related deaths occur as a result of metastasis – the dissemination of primary tumour cells through the body, resulting in the establishment of secondary tumours. In order for primary cancer cells to migrate they must invade the dense protein-rich extracellular matrix (ECM) which surrounds them. Many invasive cancer cells produce membrane protrusions, known as invadopodia, which extend into the ECM and facilitate its degradation through their enrichment in proteolytic enzymes. It has been found that digestion of the ECM is accomplished primarily by the cell surface enzyme membrane type-1 matrix metalloproteinase (MT1-MMP), allowing tunnels to be formed through which cells can navigate. Recently, it was determined that MT1-MMP must be internalized from the plasma membrane and recycled to the migration front for a cell to maintain its invasive phenotype. As well, in order for a cancer cell to migrate through areas of ECM degradation generated by MT1-MMP, it must adhere to the ECM via integrin receptors at its leading edge. Therefore, navigation through the surrounding ECM requires both MT1-MMP localized to invadopodia to degrade the matrix, as well as integrin-

anchored actomyosin contractile force. Here we show that MT1-MMP phosphorylation is dependent upon β1-integrin through the use of integrin activating and inactivating antibodies. Biochemical analyses indicate that the activation status of the epidermal growth factor receptor (EGFR) and Src kinase are also altered upon antibody treatment, pointing to their possible involvement in the MT1-MMP signaling pathway. Further analyses suggest that invadopodia formation, gelatin degradation, and the migratory phenotype of MDA-MB-231 cells are altered upon treatment with either of the integrin antibodies. These results suggest that MT1-MMP activation is in part regulated through β1-integrin signaling, leading to changes in the invasive phenotype of the cell.

Funding: NSERC

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15) *Investigating viability, metabolic activity and oxidative stress in glioblastoma cells cultured under physiological oxygen conditions*

Brianna Guild, James Uniacke*
Department of Molecular & Cellular Biology, CBS, University of Guelph

Human cell culture is widely used as a model to investigate and characterize molecular and biochemical processes. Oxygen availability, a crucial variable in human physiology, is often neglected in cell culture. Indeed, cell culture is typically performed in ambient air (21% oxygen), termed normoxia, while the documented physiological oxygenation range of human tissues, termed physioxia, is 1–11%. Several studies have suggested beneficial effects of culturing mammalian cells in low oxygen; however, to date there has been no systematic investigation of human cell cultures within the physioxic range. Here we show that culturing human primary glioblastoma cells within physioxia promotes cell viability and metabolic activity, and a decreased oxidative stress response. Our data suggests that culturing human cells in physioxia may provide a better model for making physiological inferences.

Funding: NSERC

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16) *Evaluating phage infiltration into solid tumours*

Hayden Huh, Julia Fux, Roger Chen, Marianna Foldvari, Roderick Slavcev*, Jonathan Blay*
School of Pharmacy, University of Waterloo

Colon carcinoma initiates in the epithelium of the large intestine. Bacteriophages (‘phage’) that reside in the intestinal lumen have a propensity to penetrate through the epithelium and hence, phage naturally localized to the gut may be exploited in the development of a drug delivery platform to target colorectal cancer cells. A major limitation of many anticancer therapeutics is their limited capacity to gain access to the cells in the deeper tissues of malignant tumours. Thus, as an initial approach for the development of phage in therapeutic delivery, our research aims to evaluate the infiltrative capabilities of native phages in tissues associated with colorectal cancer

both quantitatively and qualitatively. To mimic the structure of tumours in vivo, we are developing a 3D multicellular model – spheroids – that will ultimately include all of the cell states to accurately represent solid tumours. We have begun with aggregates of 3T3 fibroblast cells to model the connective tissue. To characterize the phage-fibroblast interaction, we are employing coliphage Lambda decorated by eGFP through phage display to visualize the penetrative capacity of these fluorescent phages via confocal microscopy. This experiment allows us to assess the depth of phage penetration as well as phage localization within the spheroid. Upon assessing fluorescence arising from phage infiltration, various cell layers will then be harvested and resident phage will be isolated and quantified via traditional plaque assays. The assay will be conducted both in the presence and absence of host *E. coli* to also determine whether phage propagation facilitates phage infiltration into tissue.

Funding: NSERC

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17) *Hypoxia represses E-cadherin translation and activates cadherin-22 synthesis via eIF4E2 to drive cancer cell migration, invasion and adhesion*

Nicole Kelly, Joseph Varga, Erin Specker, James Uniacke*
Department of Molecular and Cellular Biology, CBS, University of Guelph

Hypoxia is a driver of cell movement in processes such as development and tumor progression. The cellular response to hypoxia involves a transcriptional program mediated by hypoxia inducible factors, but translational control has emerged as a significant player. In this study, we demonstrate that a cell-cell adhesion molecule, cadherin-22, is upregulated in hypoxia via mTOR-independent translational control by the initiation factor eIF4E2. We identify new functions of cadherin-22 as a hypoxia-specific promoter of cell migration, invasion, and adhesion. Silencing eIF4E2 or cadherin-22 significantly impairs MDA-MB-231 and U87MG cell migration and invasion only in hypoxia, while re-introduction of the respective exogenous gene restores the normal phenotype. Cadherin-22 is evenly distributed throughout spheroids and is required for their formation and support of a hypoxic core. In human glioma specimens, cadherin-22 colocalizes with the hypoxic marker carbonic anhydrase 9. Conversely, E-cadherin translation is repressed by hypoxia and is only expressed in cells at the spheroid perimeter. Our data reveal that cadherin-22 is a hypoxia-specific cell-surface molecule that could play a role in cell adhesion and movement in development and tumor progression.

Funding: Canadian Cancer Society, Cancer Research Society, CFI

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18) *Evaluation of serum TGF- β_1 levels in canine cancers*

Changseok Kim[1], Jodi Morrison[2], Karolina Skowronski[1], J. Paul Woods[1], Brenda L. Coomber[2]*

[1]Department of Clinical Studies, [2]Department of Biomedical Sciences, OVC, University of Guelph

Transforming growth factor beta (TGF-β) is a pleiotropic cytokine that regulates cellular and physiologic processes. Alteration of TGF-β signaling pathways is frequently observed in human cancers. Increased TGF-β1 was found in the serum of human patients with various types of cancers, suggesting a potential diagnostic value for this cytokine. In this pilot study, we measured TGF-β1 levels in the serum of dogs with various types of malignant sarcomas and evaluated the potential correlation between TGF-β1 levels and outcome in canine cancer patients. Serum samples from 46 dogs diagnosed with cancer were used in this study. The cancers consisted of: mast cell tumour (14; 8 low grade, 6 high grade), osteosarcoma (11), lymphoma (5), fibrosarcoma (5), hemangiosarcoma (5), rhabdomyosarcoma (4), and GIST (2). Twelve control samples were also obtained from normal healthy dogs. All serum samples were analyzed using a commercially available ELISA kit. Log-rank test was utilized to investigate the association between circulating TGF-β1 and prognosis (PFS/OS). Median TGF-β1 values of the cancer patients and the control dogs were 26.9 ng/ml and 30.4 ng/ml (mean 36.2 ng/ml and 46 ng/ml, respectively) ($P = 0.114$). The survival analysis revealed a trend for low serum TGF- β1 and worse prognosis for dogs with osteosarcoma, and a trend for high serum TGF- β1 and worse prognosis for mast cell tumour. In conclusion, while no significant differences were observed in serum TGF-β1 levels among the studied cancer groups and control group, TGF-β1 might have prognostic value in canine osteosarcoma and mast cell tumour.

Funding: OVC Pet Trust Fund

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19) *Signalling Adaptor ShcD Suppresses Erk Phosphorylation Distal to the Ret and Trk Neurotrophic Receptors*

Hayley R. Lau, Melanie K.B. Wills, Nina Jones*

Department of Molecular & Cellular Biology, CBS, University of Guelph

The Shc family of PTB/SH2 adaptor proteins serves to connect upstream phosphotyrosine-based receptor inputs with downstream regulation of cellular effectors involved in Erk/MAPK signal transduction pathways, such as Grb2. In the nervous system, Shc proteins function to regulate neural cellular processes including cell migration, differentiation and survival. Our laboratory has previously shown that ShcD, the least characterized member, is robustly expressed throughout the nervous system, and that it is overexpressed in human gliomas. However, its involvement in neural cell signalling remains largely unexplored. We now reveal that ShcD binds activated TrkA, TrkB and Ret neurotrophic receptors predominantly via its PTB domain to suppress phosphorylation of the Erk MAPK. Concurrently, we confirm that systemic loss of ShcD in mice leads to enhanced Erk phosphorylation in the brain. We further show that ShcD suppresses Erk activation downstream of TrkB through recruitment of Grb2 to phosphorylated tyrosine residues in the central CH1 region, effectively sequestering Grb2 away from the

receptor. These findings are supported by rescued Erk activation when the ShcD PTB domain and CH1 tyrosine residues are independently mutated, thereby breaking the interaction of ShcD with Grb2 and favouring TrkB-Grb2 association. Lastly, we demonstrate that in the presence of wildtype ShcD, Erk phosphorylation can be restored through Grb2 overexpression. Activation of Erk is a key step in numerous neural cell processes, thus we propose that this novel molecular mechanism may contribute to the development of gliomas harbouring increased ShcD expression.

Funding: NSERC

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20) *Modulation of cell migration by the TGFbeta-TAZ signaling axis in canine osteosarcoma cell lines*

Anita Luu[1], Geoffrey Wood[2], Alicia Viloria-Petit[1]*
[1]Department of Biomedical Sciences, [2]Department of Pathobiology, OVC, University of Guelph,

Osteosarcoma (OSA) accounts for 85% of all primary bone tumors in canines. Despite aggressive treatment modalities a majority of canine patients develop metastasis, which is the ultimate cause of patient mortality. Previous literature has demonstrated that transforming growth factor beta (TGF β) and transcriptional coactivator with a PDZ-binding motif (TAZ) both independently and cooperatively promote cancer metastasis. However, this relationship has not been explored in canine OSA. To explore the function of TGF β -TAZ signaling in promoting cell migration, in vitro techniques involving small interfering RNA (siRNA) specific to TAZ and TGF β treatment were used. The impact of TAZ knockdown and TGF β treatment on cell migration was evaluated using the scratch-wound and transwell assay in three canine OSA cell lines: two derived from primary tumors, and one derived from the metastatic lesion. A two-way ANOVA and post-hoc Bonferroni t-test was used to determine significant differences in wound closure and cell migration. The metastatic cell line displayed similar results between both assays. TAZ-knockdown significantly impaired wound closure 8 hours and 16 hours post wounding ($p = 0.001$) and the number of migrated cells ($p = 0.018$) when compared to the control group. As these results were not observed in the two primary OSA cell lines, it suggests that TAZ may play a more crucial role in mediating cell migration during latter stages of OSA. Further analyses utilizing additional OSA cell lines and an environment that better recapitulates the tumor microenvironment are required to elucidate this relationship.

Funding: OVC Pet Trust Fund

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21) *Combined Vessel Normalization and Oncolytic Virus Therapy in the Treatment of Advanced Stage Ovarian Cancer*

Kathy Matuszewska[1], Lisa Santry[2], Byram Bridle[2], Sarah K. Wootton[2], Jack Lawler[3], Jim Petrik[1]*
[1]Department of Biomedical Sciences, [2] Department of Pathobiology, OVC University of Guelph; [3]Beth Israel Deaconess Medical Center, Harvard University, Boston MA, USA

Epithelial Ovarian Cancer (EOC) is the most lethal gynecological cancer, taking 1,750 Canadian lives per year (CCS, 2015). Often referred to as 'The Whispering Disease', EOC lacks presentation of early symptoms, forcing diagnosis at advanced stages when treatment strategies are largely ineffective. This demonstrates the need for innovative approaches to combat advanced EOC. Oncolytic virus therapy uses natural or engineered viruses that replicate in tumor cells but leave non-neoplastic tissues unharmed. The issue with oncolytic virotherapy is a phenomenon, called vascular shutdown, which leads to reduced tumor perfusion and hindered delivery of viruses. Our lab has previously demonstrated that pre-treatment with a potent anti-angiogenic molecule called 3TSR normalizes tumor vasculature in an orthotopic, syngeneic mouse model of ovarian cancer. Normalized vasculature proved to be more efficient in delivering chemotherapeutic agents to the primary tumor, thereby increasing survival of mice treated with 3TSR prior to Paclitaxel. We now investigate the efficacy of 3TSR in preventing the vascular shutdown caused by an oncolytic virus. Using an orthotopic, syngeneic mouse model of advanced EOC, we treated mice with NDV alone or following pre-treatment with 3TSR. Mice given combination treatment had significantly smaller primary tumors as well as less abdominal ascites and secondary lesions. Together, our data suggests a novel use for 3TSR when combined with oncolytic virotherapy.

Funding: OVC Scholarship & CIHR

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22) *Vectorization of anti-CTLA-4 checkpoint inhibitors in an oncolytic Newcastle disease virus as a novel strategy for targeting immunotherapies to the tumor microenvironment*

Thomas M McAusland, Lisa A Santry, Sarah K Wootton*
Department of Pathobiology, OVC, University of Guelph

Cancer immunotherapies, which employ the use of monoclonal antibodies that target immune checkpoint molecules including cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and Programmed cell death 1 (PD-1), have shown great promise in the clinic. However, systemic administration of antibodies targeting immune checkpoints, such as Ipillimumab, can lead to unwanted side effects. We hypothesize that expressing immune-checkpoint inhibitors from an oncolytic virus would localize the treatment to the tumor microenvironment and mitigate the risks inherent in systemic delivery. Newcastle disease virus (NDV) is a promising oncolytic virus with a strong safety profile due to its sensitivity to type I IFN. As such, we have used NDV as a platform to generate recombinant viruses expressing either a single-chain fragment variable Fc (scFv-Fc) or whole monoclonal antibody (mAb) against murine CTLA-4. In addition, the non-structural protein 1 (NS1) from the PR8 strain of Influenza A will be expressed from a second transcriptional cassette with the aim of increasing transgene expression. In vitro assays will evaluate the ability of NS1 to transiently reduce the IFN response and increase transgene expression in B16-F10 and CT26-LacZ tumor cell lines. In vivo studies using syngeneic models will determine the tumour neutralizing capabilities of the aforementioned NDV recombinants and allow for a comparison of the scFv-Fc vs. the mAb. Further in vivo studies using contralateral tumour models will evaluate local and distant tumour infiltrating lymphocytes.

NDV has been shown to induce a tumor-specific immune response capable of reducing distant tumours, and as such provides rationale for further combination therapy research.

Funding: private donor

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23) *Hypoxia stimulates an increase of Neuropeptide Y Y1 and Y5 receptor expression in human breast cancer cells*

Phil Medeiros, Jim Uniacke*
Department of Molecular & Cellular Biology, CBS, University of Guelph

A hypoxic microenvironment is a common physiological attribute in solid tumors. Low O_2 in tumors promotes a malignant phenotype, associated with increased proliferative, angiogenic and metastatic potential. Accumulating evidence suggests that neuroendocrine factors and their corresponding receptors may contribute to the initiation, development and progression of various cancers. Neuropeptide Y (NPY) and its receptors have been implicated as such a factor in the progression of breast cancer. Previous studies have reported the expression of the Y1R and Y5R receptor subtypes in human breast cancer cells and tumors. We have demonstrated that in breast cancer cells, NPY stimulation of Y5R increased proliferation and migration, and induced the release of vascular endothelial growth factor. In the current study, we examined the effects of O_2 concentration on NPY receptor expression in MCF-7 and MDA-MB-231 cells. Using quantitative PCR (qPCR) and Western blot, we compared Y1R and Y5R mRNA and protein expression in cells exposed to 1% (hypoxia) and 21% (normoxia) O_2. Y1R and Y5R mRNA was quantified after 1h, 3h, 6h, 12h and 24h of hypoxia. Relative to normoxic cells, we observed a significant increase in Y1R and Y5R mRNA after 3h's of hypoxia in both cell lines. Protein expression of Y1R and Y5R was examined after a 24h hypoxic exposure. Y1R increased 2-fold (MDA-MB231) compared to normoixa. Y5R expression increased 79% (MCF-7) and 55% (MDA-MB231) relative to normoxic cells. Previous work from our group has demonstrated that hypoxic cancer cells require eukaryotic initiation factor 4E2 (eIF4E2) to direct the cap-dependent translation of select mRNAs, therefore we tested whether the hypoxia-induced increases in Y1R and Y5R expression were dependent on eIF4E2. Using MDA-MB 231 cells depleted of eIF4E2, we found that hypoxic treatment had no effect on Y1R and Y5R expression compared to normoxic cells. These data provide insight into the O_2-mediated regulation of NPY receptors in breast cancer. Taken together with our previous functional NPY receptor studies, our findings provide further evidence of pathological implications of the NPY system in breast cancer. Ongoing studies will further elucidate the transcriptional and translational regulation of NPY receptors in response to hypoxia.

Funding: Ontario Ministry of Research and Innovation; Cancer Research Society

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24) *Cytotoxic Effects of Curcumin Functionalized Gold Nanoparticles in Human Prostate Cancer Cells*

Shruti Nambiar[1,2], Ernest Osei[2,3,4,5], Andre Fleck[2,5], Vahid Raeesi[1,2], Johnson Darko[2,4,5], Anthony J. Mutsaers[5], Shawn Wettig[1,6]*

[1]School of Pharmacy, University of Waterloo; [2]Department of Medical Physics, Grand River Regional Cancer Centre; [3]Department of Systems Design Engineering, University of Waterloo; [4]Department of Physics and Astronomy, University of Waterloo; [5]Department of Clinical Studies, OVC, University of Guelph; [6]Waterloo Institute for Nanotechnology, University of Waterloo

Curcumin, a natural flavonoid derived from the rhizome of turmeric, is one of the most widely reported nutraceuticals for its medicinal properties (anti-cancer, anti-inflammatory, and anti-bacterial/-viral/-fungal properties), but its poor water solubility presents a major challenge that limits the bioavailability of curcumin in vivo. One method to increase bioavailability (i.e. water solubility) is to conjugate curcumin on the surface of metal nanoparticles. In this regard, a few studies have recently reported the synthesis and potential therapeutic applications of gold nanoparticles functionalized with curcumin (or its derivatives). However, the colloidal stability and consequently, the therapeutic potential of curcumin-functionalized gold nanoparticles (cur-AuNPs) in a physiologically-relevant environment, rich with ionic-salts, serum proteins and other biomolecules, is not well understood. To evaluate the potential role of serum protein on the colloidal stability of cur-AuNPs, and their behaviour in vitro, we investigated the viability and uptake of cur-AuNPs in PC-3 cells cultured in medium supplemented with and without fetal bovine serum (FBS). Gold nanoparticles with an average diameter of 7-8 nm were synthesized and characterized using transmission electron microscopy, dynamic light scattering, ultraviolet-visible light spectrometry, and Fourier transform infra-red spectrometry. The in-vitro studies evaluated cell viability by MTT assay, and qualitative analysis of cellular uptake for different concentrations of cur-AuNPs. Viability of PC-3 cells was much higher in the presence of cur-AuNPs in 10% FBS, compared to that observed in the absence of serum after 24 and 48 h of treatment. Therefore, serum proteins may impact the therapeutic potential of these functionalized gold nanoparticles.

Funding: Telus Ride for Dad; Prostate Cancer Fight Foundation

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25) *Optimization of a technique for recovery of circulating tumour cells from the peripheral blood of colorectal and breast cancer patients*

Deep Patel[1], Mala Bahl[2], Jonathan Blay[1,3]*

[1]School of Pharmacy, University of Waterloo; [2]Grand River Regional Cancer Centre, Kitchener; [3]Department of Pathology, Faculty of Medicine, Dalhousie University, NS.

Optimizing the future clinical approach for the treatment of patients with metastatic cancers requires both new therapeutic targets and the ability to sample those cells that are disseminating from initial sites to form metastatic deposits. Existing methods to identify circulating tumour cells (CTCs) do not recover all cells that might be present in the circulation. We are examining

the different matrices that may be used to selectively capture CTCs from patients' peripheral blood. A group of 20 patients with Stage IV colorectal or breast cancer provide samples of heparinized blood at the time of their clinic visit. The blood is separated by density centrifugation on Ficoll-Paque PLUS® and CTCs recovered from the interface together with white blood cells are washed and placed on substrata composed of selected extracellular matrix (ECM) proteins including collagen, fibronectin, laminin and complex extracellular matrix. After 18h of incubation, non-adherent cells are washed away and the remaining cells are stained with immunofluorescence for tumour marker proteins. Data show differences in results depending upon the ECM proteins used as the capture substratum, with staining positive for epithelial markers EpCAM and pan-cytokeratin. Capture of CTCs using ECM-based substratum offer a different approach to tracking and characterizing cancer cells in the peripheral blood solely using EpCAM antibody-based protocols. Further work is directed toward identifying markers of disease progression such as chemokines.

Funding: CIHR

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26) *Inhibition of the Mevalonate Pathway in Transformed Fallopian Tube Epithelial Cells*

Madison Pereira, Kata Osz, Jim Petrik*
Department of Biomedical Sciences, CBS, University of Guelph

Epithelial ovarian cancer (EOC) is the most lethal gynaecological cancer and is often not detected until late stages when cancer cells metastasize into the abdomen and typically become resistant to therapy, leading to low survival rates. While much work has been done on ovarian epithelial cancer cells, recent evidence suggests that human fallopian tube epithelial (FTE) cells are responsible for the initiation of high-grade serous adenocarcinomas. In late stage disease, the tumour cells within the abdominal ascites are irreversibly re-programmed with an increased resistance to apoptosis. Majority of the genes upregulated in the aggressive ascites-derived cells (28-2 cells) have developed a gain-of-function p53 mutation and subsequent upregulation of the mevalonate pathway for a survival advantage, resulting in these cells becoming reliant upon this pathway. Simvastatin treatment to inhibit HMG CoA reductase, the rate-limiting enzyme of this pathway, has been shown to induce significant apoptosis in the 28-2 cells. We hypothesize transformed FTE cells will have developed a similar p53 mutation, with associated upregulation of the mevalonate pathway. We will evaluate the role that p53 mutations play in the reprogramming of the 28-2 and FTE cells and the upregulation of the mevalonate pathway seen in these cells. Preliminary in vitro studies demonstrated that treatment with simvastatin reduced cell viability and increased apoptosis in p53 mutant human FTE cells. As such, the use of simvastatin to inhibit the mevalonate pathway may specifically target the metastatic abdominal disease seen in advanced stage EOC and could significantly improve the ability to treat the disease.

Funding: CIHR

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27) *ICCI comparative oncology program: Clinical research studies in companion animal patients with cancer*

Vicky Sabine[1], Allison Forget[1], Kaya Skowronski[1], Michelle Oblak[1], Geoff Wood[2], Brenda Coomber[3]*, Paul Woods[1]*
Departments of [1]Clinical Studies, [2]Pathobiology, [3]Biomedical Sciences, OVC, University of Guelph

Similar to people, cancer is common in companion animals (CA) with ~1:3 dogs and 1:7 cats developing cancer and ~50% of pets >10 years old dying of the disease. Model systems are a critical component in the development of new therapies. Current cancer models (e.g. tumour cell lines, genetically engineered and/or immunodeficient mice) are valuable research tools; however, they possess shortcomings that may partly explain the poor success rate in translating basic findings to improved clinical outcomes. Many CA cancers share similar characteristics to cancers in people. Studies in CA cancer patients offer potential to fill the gap that exists between preclinical and phase I/II studies. The studies enable OVC HSC to offer further benefit to patients (e.g. novel techniques and treatment options) and in addition they provide an opportunity to obtain preclinical data for translational research relevant to human cancer. Oncology-related clinical research trials at OVC HSC are performed with the Institute for Comparative Cancer Investigation (ICCI). The ICCI combines expertise in basic cancer biology and veterinary medicine enabling an integrated approach that cannot always be matched in human research. The ICCI is the only Canadian member in the National Institute of Health-National Cancer Institute (NIH-NCI) Comparative Oncology Trials Consortium (COTC). At present, there are 12 oncology-related clinical trials recruiting patients at OVC HSC: 10 canine, 1 feline and 1 both dogs & cats (http://ovc.uoguelph.ca/icci/trials). Hence, the ICCI comparative oncology program at OVC HSC has the potential to improve the lives of both animals and people.

Funding: OVC Pet Trust Fund; The Smiling Blue Skies Cancer Fund

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28) *Temperature as a confounding variable in oncolytic virotherapy for canine melanomas*

Julia F. Saturno, Jacob P. van Vloten, Lisa A. Santry, Robert C. Mould, Sarah K. Wootton, Byram W. Bridle*
Department of Pathobiology, OVC, University of Guelph

High-grade canine melanomas are almost uniformly fatal despite aggressive interventions. The current standards of care are expensive and time consuming, making treatment an unfeasible option for many pet owners. We published a strategy to use oncolytic viruses (OVs) as booster vaccines to synergize benefits between immuno- and oncolytic virotherapies and have developed infrastructure to test this in companion animal trials. The purpose of this study was to identify an ideal OV platform for canine melanoma therapy. The oncolytic potential of Maraba virus (MG1), vesicular stomatitis virus (VSV), Newcastle disease virus (NDV) and vaccinia virus (VV) was evaluated in the patient-derived canine melanoma cell lines ICCI25cl.5, CML1 and CML10C2. Madin-Darby Canine Kidney (MDCK) epithelial cells were included as non-malignant controls. To simulate elevated normal body temperatures of canines compared to mice and humans,

account for induction of fevers in patients treated with OVs, and recapitulate the conditions of tumours located in diverse anatomical locations, efficacy was assessed across a range of temperatures. A high-throughput resazurin dye-based metabolic assay was used to assess the viability of OV-treated cells. All of the viruses demonstrated differential killing with various degrees of cytotoxicity in melanoma but not MDCK cells. At higher temperatures both MG1 and VSV lost oncolytic activity while NDV and VV remained efficacious. Through heat-adaptation, we were able to restore the replication potential of MG1 at elevated temperatures. Clinical considerations include using OVs that retain efficacy over a broad range of temperatures or utilizing heat-adaptation for temperature-sensitive viruses.

Funding: National Center of Excellence in Biotherapies for Cancer Treatment (BioCanRx), OVC Pet Trust Fund, Merial

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29) *Differentiating dendritic cells in the presence of interleukin-4 enhances their potential as vaccines*

Mankerat Singh, Byram Bridle,* Khalil Karimi*
Department of Pathobiology, OVC, University of Guelph

Dendritic cells (DCs) are professional antigen-presenting cells that can be used as vaccines to prime cancer-specific CD8+ cytotoxic T cells. However, controversy exists in the literature regarding how to differentiate DCs from bone marrow progenitor cells, with approximately half of the groups culturing them in the presence of granulocyte macrophage colony-stimulating factor (GM-CSF) alone, while the other half uses GM-CSF in addition to interleukin (IL)-4. We sought to resolve the debate by conducting a head-to-head comparison of the two methods. Bone marrow cells derived from C57BL/6 mice were cultured for seven days in the presence of 20 ng/ml of GM-CSF +/- 10 ng/ml of IL-4. Both treatments yielded DCs that were similar in terms of CD11c receptor expression. However, expression of MHC class II and costimulatory molecules (i.e. CD40 and CD80) was significantly higher when the culture was exposed to IL-4. Additionally, we observed that DCs differentiated in the presence of IL-4 had a significant increase in pro-inflammatory cytokine production (i.e. tumor necrosis factor-α and IL-6) upon stimulation with lipopolysaccharide (LPS). Furthermore, DCs cultured with IL-4, pulsed with OVA257-264 peptides, stimulated for two hours with LPS and then administered into footpads of mice, induced higher magnitude CD8+ T cell responses than DCs cultured with GM-CSF alone. Interestingly, CD8+ T cells induced with IL-4-exposed DCs, produced higher levels of interferon-γ. Currently, we are testing the hypothesis that differentiation of DCs with IL-4 supports the induction of a greater number of CD8+ T cells with a memory phenotype.

Funding: BioCanRx, Terry Fox Research Institute

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30) *25-hydroxyvitamin D concentrations in dogs with lymphoma during chemotherapy treatment*

N. Weidner[1], J.P. Woods[2], A.J. Mutsaers[1,2], J. Bayle[3], A. Verbrugghe[2]*
[1]Department of Biomedical Sciences, [2]Department of Clinical Studies, OVC, University of Guelph; [3]Royal Canin Research Centre, Aimargues, France

Studies have reported lower blood vitamin D (25(OH)D) concentrations in dogs with lymphoma compared to healthy dogs, but have not investigated 25(OH)D concentrations in lymphoma patients before and during chemotherapy. This study aimed to determine if vitamin D concentrations increased when lymphoma patients were in clinical remission. Dogs with lymphoma (n=24) presenting to the Mona Campbell Centre for Animal Cancer for treatment were enrolled. Blood samples were collected prior to treatment and at week 6 of a standard chemotherapy protocol. Remission status was also recorded. Plasma 25(OH)D was analyzed using radioimmunoassays. Baseline 25(OH)D concentrations were compared to those at week 6 using Wilcoxon signed-rank tests. Most dogs had plasma 25(OH)D concentrations within the laboratory reference range before treatment (20/24) and after remission (21/24). 20/24 dogs were in complete remission at week 6. 25(OH)D concentrations did not differ before treatment and after remission (respective means±SD= 93.8±34.2 nmol/L, 86.7±30.8 nmol/L, p=0.695), or over the same time period for the 4 dogs not in remission. (respective means±SD= 109.2±28.2 nmol/L, 110.0±21.0 nmol/L, p=1.000). Mean plasma 25(OH)D concentrations of lymphoma dogs were similar to previous reports. Although most dogs fell within the 25(OH)D laboratory reference range, researchers have suggested more work is needed to define optimal 25(OH)D concentrations. Since plasma 25(OH)D concentrations did not change, 25(OH)D concentrations may have already been sufficient in these dogs. It is worth noting that these dogs were being treated with a 25-week long chemotherapy protocol. Dogs may have needed to be in remission for longer before 25(OH)D concentrations improved.

Funding: OVC Pet Trust, Royal Canin, AAVN/Waltham

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31) *The Ontario Veterinary College University of Guelph Companion Animal Tumour Sample Bank: facilitating translational cancer research*

Paul Woods[1]*, Kaya Skowronski[1], Allison Forget[1], Vicky Sabine[1], Courtney Schott[2], Michelle Oblak[1], Geoffrey Wood[2], Brenda Coomber[3]
[1]Department of Clinical Studies, [2]Department of Pathobiology, [3]Department of Biomedical Sciences, OVC, University of Guelph

The Companion Animal Tumour Sample Bank (CATSB) in the Mona Campbell Centre for Animal Cancer at the Ontario Veterinary College has the goal of facilitating basic and translational veterinary oncology research by providing high quality samples to researchers. The CATSB is the only veterinary oncology tissue bank in Canada and is registered with the Canadian Tissue Repository Network. Sample types collected and stored at ultracold temperature are: serum, plasma, buffy coat, urine, and tissue. Tissue samples, both tumour and matched normal, are collected immediately following surgical excision and are available as flash frozen, in RNAlater, and in CryoMatrix. Tumour tissue is also formalin fixed, paraffin embedded,

sectioned, and H&E stained for quality control analysis by a pathologist. Currently, CATSB has over 1,000 cases banked. The three most prevalent canine tumour types are soft tissue sarcoma (92), osteosarcoma (90), and mast cell tumours (85), but a variety of other neoplasms have also been banked. There are currently 10 primary cell lines from canine and feline tumours available, with more in development. Researchers access samples by filling out an application form. Prospective sampling can also be tailored to suit the needs of researchers. A small cost-recovery fee is applied to enable the CATSB to continue its mission. In addition researchers can also receive patient signalment, histopathology, and follow-up data. The CATSB strives to facilitate research that will improve the lives of companion animals with cancer, with the potential to contribute to comparative human cancer research.

Funding: OVC Pet Trust Fund; The Smiling Blue Skies Cancer Fund

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32) *Exploring the role of adaptor protein ShcD in gliomas*

Manali Tilak, Nina Jones*
Department of Molecular & Cellular Biology, CBS, University of Guelph

Malignant gliomas are often characterized by two key features: misregulation of growth factor receptor-mediated signaling cascades and induction of a strong angiogenic phenotype. Specialized circuitry translates extracellular signals into cellular responses through signaling complexes comprised of receptor tyrosine kinases (RTKs), phosphatases, and adaptor proteins containing modular interaction domains. Prototypical adaptors such as the Shc (Src homology and collagen) family of four evolutionarily related proteins act as cytosolic sensors that respond upon receptor engagement. We have demonstrated that the most recently isolated member, ShcD, is overexpressed in gliomas and promotes hyperphosphorylation of EGFR in the absence of an external stimulus. Our group has also shown that phosphorylated ShcD suppresses Erk activation downstream of neurotrophic receptor TrkB. Together, these findings imply that ShcD phosphorylation serves as an agent of oncogenic events by influencing proliferative, differentiation, migratory, and possibly angiogenic signaling pathways. Computational prediction of ShcD-interacting oncogenic RTKs has garnered interest in Tie2/TEK, a receptor tyrosine kinase previously known only to be expressed in endothelial cells but recently shown to modulate cross-talk between glioma and endothelial cells in the tumor microenvironment. Here we show that ShcD binds to, and is phosphorylated by Tie2. Recent data suggests that ShcD also promotes hyperphosphorylation of Tie2 and possibly regulates signaling central to glioma progression. We explore this interaction further using U87 and U251 cells stably expressing ShcD since many low grade gliomas are prone to be driven to a more aggressive phenotype through signaling from the microenvironment coincident with enhanced angiogenesis.

Funding: NSERC

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33) *Estrogen Receptor Beta is a Novel Target in Acute Myeloid Leukemia*

Alessia Roma, Sarah Grace Rota, Paul Spagnuolo*
Department of Food Science, OAC, University of Guelph

Acute myeloid leukemia (AML) is a hematological malignancy resulting from the accumulation of poorly differentiated myeloid cells in the peripheral blood and bone marrow. It is associated with poor patient prognosis especially in the older population and suboptimal chemotherapeutics. Disease relapse is attributed to a small population of leukemic stem cells (LSCs) which are not eradicated by current chemotherapeutic options. As such, new therapies targeting these LSCs are needed to prevent persistence of disease and improve patient outcome. In this study, we identified diosmetin, a citrus flavonoid as a LSC targeting agent. Diosmetin imparted selective toxicity toward AML cells both in vitro and in vivo without affecting normal hematopoietic cells. Diosmetin exerted its cytotoxic effect through activation of estrogen receptor (ER) β which was identified here as a novel drug target towards AML. ERβ expression in AML cell lines and primary samples conferred cell sensitivity as greater sensitivity to diosmetin was observed where ERβ was highly expressed compared to lower-expressing samples. Genetic knockdown confirmed ERβ as diosmetin's target as cells with reduced ERβ expression were resistant to diosmetin. Conversely, induction of ERβ expression enhanced sensitivity to ERβ activation by diosmetin. Collectively, these studies highlight ERβ as a novel target for the treatment of AML.

Funding: Stem Cell Network

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34) *Investigating the role of Nck cytoskeletal adaptors in mammary development and breast cancer*

Adam Golding, Claire Martin, Laura New, Nina Jones*
Department of Molecular & Cellular Biology, CBS, University of Guelph

The adaptor proteins Nck1 and Nck2 are well established signaling nodes in cellular actin cytoskeleton remodeling. Although they were first identified as oncogenes nearly 25 years ago, there is scarce in vivo evidence supporting their ability to induce tumour development or metastasis. Our lab has recently shown that Nck promotes endothelial cell migration, angiogenic remodeling, and epithelial-to-mesenchymal transition (EMT), and others have reported a requirement for Nck in invadopodia formation. These processes are all correlated with invasion and metastasis of breast cancer cells. Accordingly, we have now determined that Nck1 and Nck2 are novel regulators of breast cancer progression, as well as mammary gland morphogenesis. Systemic loss of Nck2 but not Nck1 significantly delays mammary gland development, with decreases in ductal outgrowth and altered terminal end bud formation. Furthermore, we have found that Nck1 and Nck2 are both upregulated in aggressive human breast cancers, including HER2+ and triple negative subtypes. Using the MMTV-NIC transgenic mouse model of breast cancer, which allows simultaneous expression of activated HER2/ErbB2 and Cre recombinase in mammary epithelial cells, we have shown that loss of Nck1 alone impairs tumour growth, and that deletion of both Nck1 and Nck2 significantly extends survival and delays tumour onset and metastasis. MMTV-NIC mice lacking Nck2 are currently under development. These findings provide new physiological insights verifying the role of Nck as an oncogene, and they reveal its potential as a target to inhibit breast cancer.