UNIVERSITY of GUELPH

INSTITUTE FOR COMPARATIVE CANCER INVESTIGATION

11th Annual ICCI

Cancer Research Symposium

Wednesday May 30, 2018
OVC LLC 9:00-4:30

Introductory Remarks

Welcome to the 11th annual Guelph ICCI Cancer Symposium! This meeting is an opportunity to bring together cancer researchers from across campus and regional collaborators. Topics range from basic science through to clinical application. Thank you to Brenda Coomber and Tony Mutsaers for the opportunity to take over organization of the meeting this year and for all your hard work in previous years. We are very grateful to the amazing group of speakers and poster presenters who will be sharing their findings with us today. Dr. Daniel Gustafson is the 2018 Arthur Willis Distinguished speaker.

In the past 10 years we have seen relationships and collaborations develop that were made possible by these interactions and we look forward to sparking new collaborations and ideas through this year's meeting.

This symposium is made possible by funding from the Arthur Willis Visiting Professorship in Canine Oncology and support from the OVC Dean's office.

Drs Geoff Wood and Michelle Oblak
Pathobiology and Clinical Studies, University of Guelph
ICCI Assistant Co-Directors

ONTARIO VETERINARY COLLEGE

Administrative Support and Research Funding:

Thanks to Barb Gaudette, OVC Office of the Dean, for her administrative expertise and invaluable assistance in organizing this event, Hospitality Services for help with set up and refreshments, and Rachel Costin and Calvin Riley for assistance with the program and organization throughout the day.

The research projects presented here and the trainees performing these studies were collectively supported by grants, scholarships and contracts from: CIHR; NSERC; Terry Fox Research Institute; OGS; Ontario Trillium Scholarships; Cancer Research Society; Canada Research Chairs Program; OVC Pet Trust Fund; Smiling Blue Skies Cancer Fund; Guelph Food Technology Centre; OVC Graduate Scholarship; RNA Diagnostics Inc; Sheridan College; Renishaw; ADEISS; Art Rouse Cancer Biology Graduate Stipend; BioCanRx; Vanier Canada Graduate Scholarship; Brock Doctoral Scholarship; and the Stem Cell Network.

ICCI 11th Annual Cancer Research Symposium, Wednesday May 30, 2018

Morning Session: Room 1714, OVC LLC

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

9:05-9:35 Guest Speaker
Targeting Checkpoint Molecules in Veterinary Oncology
Dr. Sam Hocker; Department of Clinical Studies, OVC, University of Guelph

9:35- 10:20 Short talks from abstracts

1. *Chemosensitization effects of the potassium-sparing diuretic amiloride in canine osteosarcoma*
   Andrew Poon; Department of Biomedical Sciences, University of Guelph
2. *Considerations in fluorescent tagging of colon cancer cells in adhesion assays*
   Heather Dekker; School of Pharmacy, University of Waterloo, ON
3. *Targeting of Mitochondrial Bioenergetics by Shikonin as a Treatment for Acute Myeloid Leukemia*
   Alessia Roma; Department of Food Science, University of Guelph

10:20-10:45 ***Coffee Break and Poster Viewing*** **Room 1707 B & C, OVC LLC**

10:45-11:30 Short talks from abstracts

1. *β1 integrin signaling regulates invadopodia formation and cellular invasion in tumour cells*
   Olivia Grafinger; Department of Molecular and Cellular Biology, University of Guelph
2. *Investigating the role of Nck cytoskeletal adaptors in mammary development and breast cancer*
   Adam Golding; Department of Molecular and Cellular Biology, University of Guelph
3. *Characterizing the metabolic identities of the mammary epithelial subsets*
   Mathepan Mahendralingam; Department of Medical Biophysics, University of Toronto

11:30-12:20 Regional Keynote Speaker
What makes some tumours aggressive: modeling cancer lethality
Dr. Paul Boutros; Ontario Institute for Cancer Research, and Department of Medical Biophysics, University of Toronto

12:20- 1:45 ***Lunch and Poster Session*** **Room 1707 B & C, OVC LLC**

Afternoon Session: Room 1714, OVC LLC

1:45- 2:10 Guest Speaker
Osteolytic, osteoblastic and mixed bone metastases in a rat model: What are the effects of cancer and its treatment on the bone?
Dr. Margarete Akens; Department of Medical Biophysics, University of Toronto

2:10- 2:55 Short talks from abstracts

1. *Longitudinal flow cytometric identification and quantification of circulating tumor cells in dogs with naturally-occurring osteosarcoma*
   Dr. Tanya Wright; Department of Clinical Studies, University of Guelph
2. *Elucidating infection of stimulated leukocytes by oncolytic viruses*
   Ashley A. Ross; Department of Pathobiology, University of Guelph
3. *An ORF-virus-infected cell vaccine elicits long-term survival in an osteosarcoma lung metastasis model through NK cell activity*
   Jacob van Vloten; Department of Pathobiology, University of Guelph

2:55-3:30 ***Snack Break and Last Poster Session*** **Room 1707 B & C, OVC LLC**

3:30 - 4:30 Keynote Speaker; introduced by Dr. Paul Woods
Pharmacological Considerations in the Response of Tumors to Drug Therapy: Drug Exposure and Intrinsic Sensitivity.
Dr. Daniel L. Gustafson; Shipley University Chair in Comparative Oncology, Professor of Pharmacology & Biomedical Engineering, Director of Research, Flint Animal Cancer Center Director, UCCC Pharmacology Core, Colorado State University, USA

4:30-4:35 **Closing Remarks: Drs. Michelle Oblak and Geoff Wood**

KEYNOTE PRESENTATION

3:30 OVC LLC Room 1714

Dr. Daniel L. Gustafson, PhD

Shipley University Chair in Comparative Oncology, Professor of Pharmacology & Biomedical Engineering, Director of Research, Flint Animal Cancer Center, Director, UCCC Pharmacology Core, Colorado State University

Pharmacological Considerations in the Response of Tumors to Drug Therapy: Drug Exposure and Intrinsic Sensitivity

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Traditional cancer therapy has been based on histotype-dependent drug selection and maximum tolerated dose (MTD)-based dosing. This approach is being challenged by molecular classification of individual tumors and dosing based on biological effectiveness leading to a possible future where both drug and dose will be determined by tumor and patient specific characteristics. This presentation will review the current research ongoing in my laboratory that is studying how molecular characteristics of canine tumors can influence drug treatment and outcomes as well as how patient and tumor specific characteristics can impact drug exposure and response and warrant dose modification for optimal efficacy. This research is based on the fundamentals of dose-response that relate dose-exposure via pharmacokinetics (PK) and exposure-response via pharmacodynamics (PD). PK considerations will include the tumor uptake and intracellular distribution of hydroxychloroquine (HCQ) as an inhibitor of autophagy and a discussion of autophagy inhibition as a potential strategy in veterinary oncology based on autophagy-dependence as a PD indicator of antitumor response in canine tumor cell lines. Other PD indicators of response will include tumor gene expression signatures and prediction of response to chemotherapy in adjuvant therapy of canine osteosarcoma. The discussion will focus on drug and patient specific characteristics determining PK and PD and how these can be taken into consideration to optimize patient response to therapy.

Dr. Daniel L. Gustafson earned his B.S. in Biology from Santa Clara University in 1987 and his Ph.D. in Cell and Molecular Pharmacology and Physiology from the University of Nevada, Reno in 1992. He completed his postdoctoral training in Radiation Biology and Pharmacology at Colorado State University and the University of Colorado Health Sciences Center. Following his postdoctoral training, he joined the Center for Environmental Toxicology and Technology at Colorado State University in a junior faculty position working with Drs. Raymond S.H Yang and Melvin E. Andersen on modeling projects. Dr. Gustafson then moved to the University of Colorado School of Pharmacy as an Assistant Professor in Pharmacokinetics. At the University of Colorado, he developed a Pharmacology Core laboratory to serve investigators in the University of Colorado Cancer Center on pre-clinical and clinical pharmacology studies with an emphasis on pharmacokinetics and pharmacokinetic modeling. After eight years at the University of Colorado, Dr. Gustafson was recruited back to Colorado State University where he currently resides as Professor of Clinical Pharmacology, Shipley University Chair in Comparative Oncology, and Director of Basic Research for the CSU Flint Animal Cancer Center. He also serves as the Co-Leader for the Developmental Therapeutics Program and continues to serve as the Director of the Pharmacology Shared Resource for the University of Colorado Cancer Center. He has authored or co-authored over 120 original, peer-reviewed scientific articles in journals primarily in the field of cancer chemotherapy and pharmacology.

Past ICCI Symposium Arthur Willis Distinguished Speakers

2017	William Edward	2012	Timothy Fan
2016	Jaime Modiano	2011	Cheryl London
2015	Nicola Mason	2010	Matthew Breen
2014	Deborah Knapp	2009	Barbara Kitchell
2013	David Argyle		

GUEST SPEAKER:

9:05-9:35

Targeting Checkpoint Molecules in Veterinary Oncology

Dr. Sam Hocker; Department of Clinical Studies, OVC, University of Guelph

The innumerable genetic and epigenetic alterations that are characteristic of cancer provides a diverse antigen collection that the immune system can use to distinguish tumour cells from our normal cells. The intensity and quality of the antigen recognition is managed by a balance between co-stimulatory and inhibitory signals (immune checkpoints) after initiation of antigen recognition by the T cell receptor. Under normal physiological conditions, immune checkpoints are critical in maintaining self-tolerance; however, it is now evident that tumours can co-opt immune checkpoint pathways to suppress tumour immunity and allow tumour cell escape. With many of the checkpoint pathways being activated by a ligand-receptor interaction, they make exceptional targets to be blocked by antibodies. Currently, there are several approved immune checkpoint inhibitors licenced for the treatment of various human malignancies. With current clinical approaches to canine cancer consisting primarily of surgical, radiation, and chemotherapeutic interventions, immune checkpoint blockade (ICB) is an appealing therapeutic selection in veterinary oncology.

REGIONAL KEYNOTE SPEAKER:

11:30-12:20

What makes some tumours aggressive: modeling cancer lethality

Dr. Paul Boutros; Ontario Institute for Cancer Research, and Department of Medical Biophysics, University of Toronto

Human cancers are remarkably variable in their initial presentation. They can arise in almost every organ of the body, at almost any age. Even within a single tumour type, they vary dramatically in morphological characteristics, like their size, location within an organ and cellular structure. These larger changes are reflected in, and presumably at least partially driven by, differences in the specific somatic mutational characteristics between tumours. Indeed, while individual tumours can harbour tens to hundreds of thousands of single-nucleotide mutations, in many tumour types, the median number shared by any pair of cancers is zero. These morphological and molecular heterogeneities are mirrored by a remarkable heterogeneity in clinical outcomes. Many cancers are cured by definitive local therapy – most commonly surgery or radiotherapy. Other tumours, however, are highly aggressive, likely as a result of some combination of selection of specific subclonal populations and/or their adaptation to new selective pressures. This diversity in clinical response leads to both over-treatment of tumours which are not aggressive, and under-treatment of aggressive ones. As a result, there remains an urgent need to understand which tumours are highly aggressive, and which are not, so that therapies can be tailored to individual patients.

We undertook a systematic evaluation of the origins of differential tumour aggressivity. Initially using prostate cancer as a model tumour type, we evaluate the relative contributions of somatic mutational features, epigenomic features, transcriptomic features and proteomic features. Ultimately we discover that aggressivity is a complex function of all of these, and is driven not only by snapshot of mutations present at diagnosis, but also by the evolutionary trajectory upon which the tumour is embarking. Finally, we evaluate why evolutionary trajectories differ within and between a broad range of cancer types, and offer suggestions for the derivation of evolutionarily-aware biomarkers.

The highly divergent outcomes of tumours when they are localized and curable remains the single most pressing clinical challenge in almost all tumour types. High-throughput molecular assays have shed significant light on this problem, and we are at the cusp of a transformation from discovery-science to validation- and implementation-science in this space.

GUEST SPEAKER:

1:45-2:10

Osteolytic, osteoblastic and mixed bone metastases in a rat model: What are the effects of cancer and its treatment on the bone?

Dr. Margarete Akens; Techna Institute at the University Health Network, Department of Medical Biophysics, Department of Surgery, Spine Program and Collaborative Program in Musculoskeletal Sciences, University of Toronto

Bone is a preferred site for cancer metastases. Depending on the primary cancer the lesion in the bone are osteolytic (bone resorbing), osteoblastic (bone forming) or mixed (osteolytic and osteoblastic). Pre-clinical murine models are used to study the effect of metastases on bone quality and properties as well as cancer treatment effects. We have established and extensively studied an osteolytic and mixed osteoblastic/osteolytic metastases model in the rat and recently established an osteoblastic metastases model. The metastases are established by the intra-cardiac injection of 1.5-2.0x10^6 cells (HeLa (human adenocarcinoma cells) cause osteolytic lesions; ACE-1 (canine prostate carcinoma) cause mixed lesions; ZR-75-1 (human breast carcinoma) cause osteoblastic lesions). The development of bone metastases in the appendicular bones and vertebrae takes approximately 2 weeks with the HeLa and ACE-1 cells and around 3-4 months with the ZR-75-1 cell line.

SHORT TALKS FROM SUBMITTED ABSTRACTS

9:35-10:20 Morning Session

Chemosensitization effects of the potassium-sparing diuretic amiloride in canine osteosarcoma

A.C. Poon[1], J.M. Inkol[1], A.K. Luu[1], A.J. Mutsaers[1,2]
[1]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph
[2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph

Canine osteosarcoma (OSA) is an aggressive malignancy that typically presents within the appendicular skeleton. While chemotherapy delays metastasis, most dogs succumb to this disease within one year of diagnosis. Comparable to the evolution of multidrug resistance transporters, many chemotherapeutics become sequestered within acidic lysosomes. Furthermore, the high glycolytic capacity of cancer cells also contributes to microenvironmental acidosis, fostering immune evasion and perpetuating chemoresistance. A pilot study in companion animals suggested that proton pump inhibitors are potential anti-cancer agents in OSA. Among this class of inhibitors is amiloride, a well-tolerated potassium-sparing diuretic for heart failure treatment in dogs. Assessments of cell viability, apoptosis and metabolism were performed in three canine OSA cell lines after amiloride treatment, and pharmacological synergism with doxorubicin and carboplatin was calculated using the combination index (CI). Amiloride strongly synergized with doxorubicin (CI < 1) to reduce viability in primary and metastatic canine OSA cells. Significant increases in early apoptosis ($p < 0.0001$) were observed with combination treatment, compared to treatment with amiloride or doxorubicin alone. Amiloride treatment also upregulated p53-mediated apoptosis and downregulated Akt-specific activation in a dose-dependent manner. Finally, Seahorse metabolic profiling of OSA cells treated with amiloride revealed significant decreases in extracellular acidification rates (ECAR) ($p < 0.01$) and coupling efficiency ($p < 0.05$), leading to reduced maximal respiration. Taken together, the well-known safety profile of amiloride and its potential for synergism with doxorubicin may justify drug repurposing as an oncology drug for clinical trial evaluation in canine OSA.

Funding: OVC Pet Trust Fund, OVC Scholarship

Considerations in fluorescent tagging of colon cancer cells in adhesion assays

H. Dekker[1] and J. Blay [1,2]
[1]School of Pharmacy, University of Waterloo, ON
[2]Department of Pathology, Faculty of Medicine, Dalhousie University, NS

Drug resistance in colon cancer limits treatment options and contributes to the poor survival of patients with late-stage metastatic colon cancer. The acquisition of drug resistance is accompanied by changes in cell migration, elaboration of proteases and adhesion to the extracellular matrix (ECM), making the cancer cells behave more aggressively. We have been examining the differences in cellular ECM interactions and adhesion, between parental colorectal cancer cell lines (HT-29 and HCT116) and their counterparts that we have first made resistant to the active metabolite of the chemotherapeutic drug irinotecan (Camptosar®). In these studies our cells were first stained with the cell-entrapped fluorochrome dye, 5-chloromethylfluorescein diacetate (CMFDA, CellTracker™ Green). We noted that the drug-resistant counterparts of our colorectal cancer cell lines exhibited greater fluorescence if stained with this agent, and hypothesized that this could be due to altered

enzymatic processing of the fluorochrome substrate. The primary enzyme activities responsible for cellular processing of CMFDA are carboxylesterase and glutathione S-transferase, both of which are involved in the metabolism of irinotecan to its active metabolite SN-38. Specific enzyme activity assays were therefore performed to compare the activity levels of carboxylesterases and glutathione S-transferase between our drug-resistant and drug-sensitive cell lines. We report on the differences that we have observed and the relevance of our findings to the use of CellTracker™ Green and comparable cleavable, cell-entrapped markers used in assays of cell adhesion and distribution in both in vitro and in vivo models.

Funding: NSERC

Targeting of Mitochondrial Bioenergetics by Shikonin as a Treatment for Acute Myeloid Leukemia

A. Roma[1], P. Spagnuolo[1]
[1]Department of Food Science, University of Guelph

Acute myeloid leukemia (AML) is a hematopoietic malignancy that results from the accumulation of undifferentiated or poorly differentiated myeloid cells in the peripheral blood and bone marrow. Limited therapeutic options which have remained virtually unchanged for the last 40 years contribute to the unfavourable outcomes associated with this disease. As such, novel therapeutic targets and options need to be investigated to ameliorate patient prognosis Through a screen consisting of almost 300 nutraceuticals (food derived bioactive compounds), we identified shikonin, a napthoquinone derived from the purple gromwell as an anti-leukemic agent. Shikonin induced cytotoxicity in a panel of leukemic cell lines at an EC-50 as low as 0.6uM and preferentially targeted the clonogenic growth of primary leukemia cells while sparing normal hematopoietic progenitor cells. In a murine model of human AML, treatment with 2mg/kg shikonin significantly reduced the ability of primary AML cells to engraft in the bone marrow without causing overt toxicity. Shikonin also caused a rapid increase in whole cell and mitochondrial reactive oxygen species (ROS). This oxidative stress was essential to shikonin-induced cytotoxicity as co-incubation with N-acetylcysteine abrogated death. Shikonin interfered with mitochondrial bioenergetics by inhibiting oxidative phosphorylation in leukemia cells through hindrance of the electron transport chain. Together, these results highlight shikonin's ability to selectively target AML and warrant the further investigation of shikonin as an electron transport chain-targeting agent.

Funding: Stem Cell Network

10:45-11:30 Second Morning Session

β1 integrin signaling regulates invadopodia formation and cellular invasion in tumour cells

O. Grafinger[1], T. Stirling[1], M. Coppolino[1]
[1]Department of Molecular and Cellular Biology, College of Biological Sciences, 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), forming tunnels 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. Previous work in our lab has shown that endocytosis of the enzyme from the cell surface is dependent on a phosphorylation event on its cytoplasmic domain. Here we show that phosphorylation of MT1-MMP is a downstream result of a signaling cascade initiated by β1-integrin receptor adhesion to the ECM. Through the use of β1 integrin-specific activating and inactivating antibodies we have determined that signaling by this adhesion receptor has a direct effect on cellular migration and invasion, which may be a result of an increase in invadopodia formation dynamics. These results suggest that cellular invasiveness is regulated in part by β1 integrin signaling, and that this may be due to an increased rate of MT1-MMP recycling within the cell.

Funding: NSERC

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

A. Golding[1], C. Martin[1], L. New[1], J. Ursini-Siegel[2], R. Moorehead[3] and N. Jones[1]
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[2] Lady Davis Institute for Medical Research, McGill University, Montréal, Québec, Canada
[3] Department of Biomedical Science, University of Guelph, Guelph, Ontario, Canada

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 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. We have now determined that Nck1 and Nck2 are novel regulators of breast cancer progression, as well as mammary gland morphogenesis. Systemic loss of Nck1 or Nck2 significantly alters mammary gland development dynamics with changes in ductal outgrowth, branching area, and 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 deletion of both

Nck1 and Nck2 significantly extends survival and delays tumour onset and metastasis. Protein analysis of tumours lacking Nck1 and Nck2 shows changes in expression of FAK, B1-Integrin, and paxillin which are primary mediators in focal adhesion signaling dynamics implicated in breast cancer. 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.

Funding: Canada Research Chairs Program

Characterizing the metabolic identities of the mammary epithelial subsets

M. Mahendralingam[1], A. Casey[2], R. Khokha[1, 2]
[1]Department of Medical Biophysics, University of Toronto
[2]Princess Margaret Cancer Center, University Health Network

Cancer hijacks the metabolic network of the normal cell it originated from. Unfortunately, there is little known about the metabolism of the mammary stem and progenitor cells, the putative cell(s)-of-origin for breast cancer. The mammary epithelial hierarchy is composed of two lineages: luminal and basal, enriched for luminal progenitor (LP) and mammary stem cells (MaSC), respectively. We sought to characterize the metabolic identities of the mammary stem and progenitor cells. To do so, we performed metabolomics on the basal and LP compartments. In our initial run, we identified 90 metabolites ranging from various metabolic pathways such as glycolysis, Krebs Cycles and several amino acids. Strikingly, basal cells demonstrated an enrichment for glycolytic metabolites. Unsupervised hierarchical clustering grouped metabolic proteins according to cell type and progesterone treatment. We then tested the efficacy of metabolic inhibitors using the 2D colony-forming cell (CFC) assay. In line with the metabolomics data, glycolytic inhibitors only decreased colony formation in basal cells. Whereas OXPHOS inhibitors only decreased colony formation in the luminal compartment. Transmission electron microscopy (TEM) analysis and intracellular flow cytometry demonstrated lineage-specific mitochondrial morphology and characteristics (total level, ROS). This work provides preliminary data highlighting the unique metabolic identities of the mammary stem and progenitor cells. Future work will be geared towards treating breast cancers by targeting these lineage-restricted metabolic dependencies.

Funding: CIHR

2:10-2:55 Afternoon Session

Longitudinal flow cytometric identification and quantification of circulating tumor cells in dogs with naturally-occurring osteosarcoma.

TF. Wright[1], BA. Brisson[1], A. Tiessen[1], CR. Belanger[1], GA. Wood[2], D. Bienzle[2]
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[2]Department of Pathobiology, Ontario Veterinary College, University of Guelph

Detection of early metastasis in dogs with osteosarcoma (OSA) is limited by the sensitivity and specificity of available imaging modalities. Enumeration of circulating tumor cells (CTCs) in people has shown that detection and quantification of CTCs throughout the course of disease correlates well with cancer malignancy and stage. The objectives of this study were to devise a method for detecting OSA CTCs in dogs, and to prospectively apply this assay longitudinally to blood samples from clinical OSA patients. A flow cytometry protocol was developed for detection of OSA CTCs and used to analyze peripheral blood from clinical OSA patients prior to, and following limb amputation, and at each chemotherapy treatment and thoracic radiographic rechecks until the development of metastasis or euthanasia. Results of this study revealed that CTCs can be differentiated from other cells in blood using the developed flow cytometry protocol. Twenty-eight OSA patients with no evidence of pulmonary metastasis were enrolled. CTCs were identified prior to, and or at some point following amputation and chemotherapy. Thus far, 17/28 dogs have developed metastasis and 16 have died. When elevated prior to amputation, CTC numbers declined following amputation and chemotherapy to a near negative range. A 5 to 50 fold spike in CTCs numbers was identified 0 days to 58 days prior to radiographic recognition of pulmonary metastases in 15/17 dogs. To our knowledge this is the first study to establish such an assay in dogs with neoplasia and to attempt to assess clinical utility for use in canine patients.

Funding: OVC Pet Trust

Elucidating infection of stimulated leukocytes by oncolytic viruses

A.A. Ross[1], W. AuYeung[1], J.J. Petrik[2], S.K. Wootton[1*], B.W. Bridle[1*]
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[2]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON

An area of oncolytic virotherapy that requires additional research involves determining the optimal dosing frequency. Researchers must consider whether the goal of oncolytic virus (OV) administration is virocentric or immunocentric. If the therapy is virocentric, the goal is to administer the maximum concentration of virus frequently to maximize infection and oncolysis of cancer cells. However, if the goal of OV administration is immunocentric, the goal is to induce an *in situ* vaccine effect. Reducing the number of professional antigen-presenting cells would have implications for immunotherapies, because these innate cells are a crucial link for activating tumour-specific T cells and initiating a memory response. The aim of this study was to determine if stimulating leukocytes with OVs predisposes them to subsequent infection with OVs and, ultimately, death. *In vitro* studies demonstrated that pre-treating splenocytes and bone marrow cells with the immune cell activators phorbol 12-myristate-13-acetate and ionomycin in combination with the OVs vesicular stomatitis virus, vaccinia virus, and Newcastle disease virus led to elevated rates of infection. The impact of these dosing regimens on natural killer cells and CD8+ cytotoxic T cell-mediated responses was

quantified by flow cytometry. The administration of repeated doses in a murine model demonstrated that increased leukocyte infection also occurs *in vivo*. Moreover, multi-dosing with a VSV-vectored booster vaccine led to a significant decrease in survival rates in melanoma tumour-bearing mice as compared to a single boost. These results demonstrate that optimal dosing frequencies should be carefully evaluated before oncolytic virotherapies enter clinical trials to improve survival rates.

Funding: Terry Fox Research Institute, BioCanRx, NSERC, OVC Pet Trust Fund and the Smiling Blue Skies Cancer Fund, Vanier Canada Graduate Scholarship, and Brock Doctoral Scholarship.

An ORF-virus-infected cell vaccine elicits long-term survival in an osteosarcoma lung metastasis model through NK cell activity

J.P. van Vloten[1], R.C. Mould[1], J.C. Ingrao[1], J.J. Petrik[2], G. McFadden[3], S.K. Wootton[1*,] and B.W. Bridle[1*]

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[*]contributed equally

Osteosarcomas are bone cancers that most commonly affect medium and large breed dogs. They often metastasize to the lungs leading to fatality. Patients with osteosarcomas usually have tumours removed, providing a source of tumor tissue and an opportunity to generate personalized autologous cancer vaccines with the goal of treating metastatic disease. Here, we develop an infected cell vaccine (ICV) platform centered around Orf virus (ORFV), a highly immunogenic and oncolytic poxvirus, to treat osteosarcoma lung metastases. The syngeneic K7M2 murine osteosarcoma model was employed, which, when delivered intravenously, establishes lung tumors that prove fatal in approximately 30 days. We demonstrated that ORFV can infect and kill K7M2 cells *in vitro* by resisting a robust type I interferon response that renders other oncolytic viruses, such as vesicular stomatitis virus, ineffective. ICVs were generated by infecting K7M2 cells *in vitro* with ORFV, and then delivering them intravenously to mice previously challenged with K7M2 cells. ICV delivery as late as 16 days following tumor challenge led to a significant extension in survival and resistance to re-challenge. Histology of the lungs of ICV-treated long-term survivors found no evidence of structural damage or influx of inflammatory cells, indicating that intravenous delivery of the ICV was well tolerated. To determine the immune effector cells important for efficacy, we used monoclonal antibodies to deplete NK cells, $CD4^+$ and $CD8^+$ T cells alone as well as $CD4^+$ and $CD8^+$ T cells in combination in tumor-bearing mice, and then delivered the ICV. Therapeutic efficacy was completely abolished in mice depleted of NK cells, indicating an important role for this innate immune cell type. Future experiments aim to further explore the kinetics and effector phenotype of NK cells responding to the ICV.

Funding: Terry Fox Research Institute and the NSERC

LUNCH & POSTER SESSION 12:20-1:45
OVC LLC Room 1707 B & C
Posters will be displayed all day; authors please attend your poster 12:45-1:45

POSTER ABSTRACTS

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1) Effects of Metabolic Modulators on Survival and Oxygen Consumption in Leiomyosarcoma Cells.

B. L. Coomber[1], H. Adler[1], K. McLaughlin[1], A. J. Mutsaers[1,2]
[1]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph
[2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph

Leiomyosarcomas are malignant tumours of smooth muscle origin. This cancer often has a hyperactivated AKT-mTOR pathway, and mTOR inhibitors such as Everolimus (EV) show some clinical utility in this otherwise chemoresistant disease. One of the emerging hallmarks of cancer is a reliance on altered cellular metabolism, and energy stress induced by metabolic modulating compounds such as 3-bromopyruvate (3BP) and Metformin can induce anticancer effects. Here we investigated the impact of EV, 3BP and Metformin, alone and in combination, on cell viability and metabolism in human leiomyosarcoma cells. The uterine leiomyosarcoma cell line SK-LMS-1 was cultured in DMEM containing 10% FBS, plus or minus 1 mM sodium pyruvate, and cells were treated with LC_{50} doses of EV, 3BP and Metformin. Treated cells were subjected to a crystal violet assay to determine growth and survival, and oxygen consumption measurements were obtained with the Seahorse Xfe24 analyzer using the Mito Stress Test kit. Treatment with 3BP enhanced cell growth in the presence of pyruvate, and showed synergistic inhibition of growth in combination with EV. This same combination induced modest inhibitory effects on cellular respiration as measured by the Mito Stress Test. Metformin alone had no impact on cell proliferation, but strongly inhibited non-mitochondrial oxygen consumption, basal respiration, maximal respiration and ATP production. Metformin in combination with 3BP produced significant synergistic inhibition of cellular respiration. Taken together, our findings support the possibility that non-cytotoxic metabolic modulators may synergistically affect cellular metabolism and O_2 consumption, leading to an anticancer effect for uterine leiomyosarcoma.

Funding: Guelph Food Technology Centre

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2) Targeting the PI3K/mTOR pathway in combination with chemotherapy in a naturally-occurring model of osteosarcoma

R. Hummel[1], C. Baldwin[1], J. Morrison[1], R. Bojinca[1], A. Poon[1], A. Mutsaers[1,2]
[1]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada
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Osteosarcoma is the most common primary bone tumour in both humans and dogs and is in need of novel treatments for metastatic disease. The PI3K/mTOR pathway is a promising therapeutic target

for osteosarcoma that, when suppressed, may improve response to chemotherapy. Furthermore, dual inhibition of this pathway may lead to greater anti-tumour effects. In this study, three canine osteosarcoma cell lines were treated with inhibitors of PI3K (LY294002), mTOR (rapamycin, everolimus) or dual PI3K/mTOR (GSK2126458) alone, and in combination with chemotherapy (doxorubicin, carboplatin). Viability was assessed using a resazurin assay and results were categorized using the combination index. Western blotting was performed to determine drug effects on PI3K/mTOR pathway activation. Most drug combinations showed synergism and decreased cell viability compared to either inhibitor or chemotherapy alone, and everolimus (mTORC1 inhibitor) showed the greatest synergism with doxorubicin. In contrast to previous literature, the dual PI3K/mTOR inhibitor GSK2126458 showed the least synergism in the doxorubicin group. However, when tested with carboplatin, dual PI3K/mTOR inhibition showed the greatest reduction in viability. Western blots revealed decreased phosphorylation of mTOR and downstream molecules such as P70S6K with combination treatment. These results in canine osteosarcoma cells show that mTOR inhibition had a greater synergistic effect with doxorubicin, but dual PI3K/mTOR inhibition was most effective when combined with carboplatin. Chemotherapy-related differences in response to PI3K/mTOR targeting may be relevant to the design of future clinical trials.

Funding Source: OVC Pet Trust

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3) Investigating the Role of Nkd1 in Wnt Signaling using CRISPR generated Knockout Zebrafish

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The Wnt signaling pathway is an essential developmental pathway that controls a variety of different processes ranging from cell proliferation to body axis patterning. It involves binding of an extracellular ligand to a receptor, after which cytoplasmic β-catenin is stabilized. This transcriptional co-activator then translocates into the nucleus to allow for transcription of Wnt target genes. Perturbations in this pathway have implications in several diseases such as osteoporosis, mental disorders and most notably, colorectal cancer in which 90% of the cases involve mutations leading to a constitutively active state. Being highly conserved in nearly all metazoans, the pathway is regulated via several mechanisms to ensure homeostatic levels. One of the ways this is achieved is by using target genes as antagonists, thereby creating a negative feedback loop. The Naked Cuticle Homolog 1 (Nkd1) is an obligate Wnt signaling target gene that has been shown to interact with cytoplasmic β-catenin to attenuate the Wnt pathway; however, evidence for this effect is predominantly seen in cases where the pathway has been over-activated. The advent of the CRISPR/Cas9 system provides an excellent opportunity to further characterize Nkd1's role in Wnt signaling by creating genetic null organisms. Using this approach, complete *Nkd1* knockout zebrafish will be generated after which the subsequent effect on the phenotype, target gene expression, and cytoplasmic β-catenin will be determined. Based on the known characteristics of Nkd1, I propose that the effects of *Nkd1* knockouts will only be seen in cases where the intensity of Wnt signaling is over stimulated.

Funding: NSERC

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4) The ShcD neuronal adaptor protein enhances the oxidative stress response of EGFR

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Neurons are associated with high metabolic demand, and an imbalance in the production or clearance of reactive oxygen species can lead to oxidative stress (OS) and neuronal damage. To combat the detrimental consequences of OS in the brain, neurons have established anti-stress mechanisms including activation of the EGFR, which in turn is implicated in tumorigenesis. We have recently reported that the neuronal adaptor protein ShcD is upregulated in human gliomas along with EGFR, and that it facilitates ligand-independent phosphorylation of the EGFR, similar to the effect of peroxide. We have now determined that ShcD synergizes with OS to modulate EGFR phosphorylation. Using the COS1 model cell system, we demonstrate that ShcD-induced EGFR phosphorylation is enhanced following peroxide-induced OS, and that this effect is dependent on the interaction between ShcD and EGFR. As the related adaptor ShcA has previously been implicated in OS signaling via its amino-terminal Collagen Homology 2 (CH2) region, we investigated whether this comparable region present within ShcD might harbour OS-response elements. Using CH2 truncation mutants, we show that loss of this region disrupts EGFR phosphorylation under conditions of induced OS. Lastly, we profiled the response of EGFR following peroxide-induced OS in SH-SY5Y human neuroblastoma cells stably expressing ShcD and found that ShcD increases OS-mediated phosphorylation of the EGFR when bound to the receptor. Our findings reveal a novel role for the ShcD adaptor in regulating OS signaling in neuronal cells.

Funding source: NSERC

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5) ISGylation is a Hypoxia-Induced Pathway that Attenuates Activity of the HIF-2α Transcription Factor

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The tumor microenvironment is characterized by chronic hypoxia (low oxygen tension) as cancer cells rapidly outgrow their blood supply. Many cancers take advantage of a gene program mediated by transcription factors hypoxia-inducible factors (HIF) 1α and 2α that target genes containing hypoxic response elements (HREs) in their promoter region. These transcription factors play an important role in tumorigenesis by promoting vascularization through the expression of angiogenic factors such as *EPO* and *VEGF*. Post-translational modifications offer a powerful tool to rapidly alter HIF-1α and -2α activities in response to hypoxia. Interferon-stimulated gene 15 (ISG15), a ubiquitin-like modifier, has been shown to down-regulate HIF-1α activity in hypoxia, however, there is no information regarding the effects of ISGylation on HIF-2α. HIF-2α is a target of interest as it has been implicated in immune escape, and a more aggressive phenotype in developing tumors. Using the colorectal cancer model cell line HCT116, our data suggests ISG15 is a suppressor of HIF-2α transcriptional activity. Overexpression of the ISGylation system enhanced HIF-2α turnover in hypoxia. Furthermore, qPCR analysis revealed that HIF-2α target transcripts decreased in the presence of ISGylation in hypoxia. Interestingly, the ISGylation system has been identified to contain HREs. qPCR analysis suggests expression of ISG15, UBE1L, and UBCH8 expression is induced in hypoxia, whereas HERC5 is not. Therefore, ISGylation likely participates in hypoxia-mediated regulation of HIF-1α and HIF-2α-mediated transcription. This work highlights ISG15 as a regulatory modifier of HIF-2α transcriptional activity in cancer cells, and provides insight into a potentially new avenue for therapeutic treatment.

Funding Source: NSERC

6) Branched Chain Amino Acid Transaminase 1 in Claudin-low Breast Cancer

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Breast cancer, the most commonly diagnosed cancer in women, can be classified into five distinct subtypes. One subtype, claudin-low breast cancer, accounts for approximately 7% of the breast cancer cases and these tumors are aggressive and highly metastatic. RNA sequencing of human claudin-low breast cancers by other groups and RNA sequencing of a murine claudin-low mammary tumor cell line by our group has revealed that *Bcat1* is significantly up-regulated in this breast cancer subtype. *Bcat1* regulates the metabolism of branched chain amino acids and has been linked to numerous pathologies including heart disease, diabetes, and cancer. Based on this data we hypothesized that the expression of *Bcat1* in claudin-low mammary tumors is driving the aggressive and metastatic nature of this cancer subtype and disrupting *Bcat1* will deter these features. Elevated expression of *Bcat1* in the murine claudin-low cell line RJ423, compared to the murine luminal mammary tumor cell line RJ345, has been confirmed at the mRNA and protein level. *Bcat1* has been transiently down-regulated ~70% in RJ423 cells using siRNA and this suppression of *Bcat1*, contrary to the anticipated result, showed no effect on proliferation based on phospho-histone H3 immunofluorescence. RJ423 stably expressing *Bcat1* shRNA and RJ345 stably expressing a *Bcat1* expression vector are currently being selected. Further *in vitro* and *in vivo* studies will utilize these stably transfected cell lines. This study will determine whether further investigation into the effects of *Bcat1* on claudin-low human breast cancer is prudent and if *Bcat1* may be used as a therapeutic target.

Funding: CIHR, OVC Scholarship

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7) Investigating the Mechanism of Avocatin-B in Acute Myeloid Leukemia Cell Death

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Acute myeloid leukemia (AML) is a devastating blood cancer that prevents functional red blood, immune, and platelet cells from developing. AML resistant to induction chemotherapy, the primary anti-AML regimen, has resulted high relapse rates in adult patients, leading to low survival rates. Unlike healthy blood cells, AML cells are more dependent on fatty acid oxidation, a process that allows the cancer to utilize fat as a fuel. In contrast to current chemotherapeutics which also harm healthy blood cells as well as cancer cells, future therapeutics targeting fatty acid oxidation may allow for selective elimination of cancer cells, thus improving patient quality of life. Avocatin-B is an avocado-derived mixture of two fat-like molecules found to induce selective AML death, while sparing healthy blood cells. My current project has found that one of these fat-like molecules slows fatty acid oxidation, starving AML cells of vital nutrients required for the survival and rapid expansion of the disease. Fatty acid oxidation is a four step process that breaks down fat into useful biomolecules critical to AML survival and propagation. The future objectives of my project will be to determine what aspects of avocatin-B's structure allow it to slow FAO and which of the four steps of FAO is being hindered by avocatin-B. Completion of these goals are critical to developing avocatin-B into a novel chemotherapeutic that selectively targets AML, expanding current treatment options.

Funding: Ontario Graduate Scholarship

8) Inferring the glioma-associated transcriptional regulatory network of adaptor protein ShcD: Analysis of the proximal promoter

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Gliomas are highly infiltrative heterogeneous tumors with increased frequencies of genetic and epigenetic alterations resulting in increased mortality rates. Dysregulation of signaling pathway components is a major contributor to their occurrence and progression. Members of the Shc family of phosphotyrosine adaptor proteins serve as platforms that bridge ligand-activated receptor tyrosine kinases with downstream effectors to form active signaling complexes. However, the more recently isolated member, ShcD, dodges the realm of canonical Shc signaling by promoting ligand-independent hyperphosphorylation and sequestration of EGFR as previously shown by our group. We hypothesized that this non-canonical behavior may be responsible for glioma occurrence and the examination of its regulatory architecture shall highlight its role as an atypical tumorigenic signaling adaptor. Non-coding regulatory elements contribute to disease through dysregulation of gene expression. Evidently, we observed highly variable expression values for *ShcD* mRNA in malignant gliomas. We, therefore, focused on a 1025 bp region upstream of *ShcD* transcriptional starting site (TSS) predicted by *in silico* analysis of evolutionarily conserved sequences. Reporter analysis of genomic fragments of varied lengths revealed a region 435-596 bp upstream of *ShcD* TSS to be the minimal promoter in HEK293T and U87 cells. We also identified a repressive element between the minimal promoter and TSS in HEK293T cells. Computational analysis of transcription factor binding sites identified consensus sites for several key stem cell regulatory factors which are also implicated in gliomas such as SOX9, NANOG, REST, and STAT3. Ongoing TF overexpression/knockdown experiments will help decode the functional role of these regulatory elements.

Funding: NSERC

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9) Innate Immune Responses to Recombinant Vesicular Stomatitis Virus: Immunosuppressive Neutrophils

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Oncolytic viruses (OVs) are a relatively novel class of therapeutic agents for the treatment of cancers. The anti-tumour function of OVs is reliant on host immune stimulation. However, host antiviral responses against OVs is a major barrier to OV-based cancer immunotherapy. Neutrophils are the first innate cells recruited to sites of inflammation and a growing body of evidence indicate their contribution in tumorigenesis. We investigated innate immune responses and neutrophil trafficking in mice infected with a highly attenuated recombinant strain of vesicular stomatitis virus (rVSVΔm51). C57BL/6 mice received intravenous injections of rVSVΔm51 at doses ranging from $1x10^5$ to $1x10^9$ pfu. Flow cytometry was used to define immunological cell subsets and detect secreted cytokines at a single-cell level. rVSVΔm51-infected mice had elevated levels of cytokines in serum and an increased frequency and number of neutrophils in their lungs, compared to controls. *In vivo* depletion of neutrophils led to further elevation of pro-inflammatory cytokines in

plasma that was not due to viral burden. Furthermore, neutrophils upregulated their expression of PDL-1 in response to infection with rVSVΔm51. Our findings suggest that neutrophils that are mobilized into inflamed sites exhibit immunosuppressive effects on antiviral cytokine responses during rVSVΔm51 infection. Future studies will test these findings in tumour-bearing mice.

Funding: NSERC Discovery Grant and Ontario Trillium Scholarship

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10) Strategies to enhance the Therapeutic potential of oncolytic Newcastle disease virus

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The avian paramyxovirus, Newcastle disease virus (NDV), is a potent oncolytic virus that has been shown to be safe and effective in a variety of preclinical cancer models as well as in human clinical trials. NDV preferentially replicates in and lyses tumor cells, while sparing normal cells. In addition, NDV possesses strong immunostimulatory properties that can overcome cancer-induced immunosuppression and generate effective anti-tumor immune responses. Recombinant NDVs (rNDVs) have been engineered to encode therapeutic transgenes that augment the virus's oncolytic and immunostimulatory properties. However, the oncolytic efficacy of NDV is negatively impacted by tumors that retain intact antiviral signalling and sensing capabilities. The objective of this research was to evaluate the use of vector-mediated or viral sensitizer-mediated suppression of host antiviral responses to enhance therapeutic transgene expression and ultimately, the antineoplastic properties of NDV. To achieve this goal, we generated a rNDV in which the antiviral antagonist from influenza virus NS1, was inserted as a separate gene cassette. NDV-GFP-NS1 infection of murine tumor cell lines results in markedly enhanced syncytia formation and GFP transgene expression, suppression of the type I IFN response and delayed cytotoxicity in comparison to the NDV-GFP parental virus. These results indicate that co-expression of NS1 may enhance therapeutic transgene expression and increase virus replication in tumors. Additionally, we endeavored to identify viral sensitizers (VSe) that function to specifically enhance NDV-mediated transgene expression and oncolysis in murine tumor cell lines. We found that depending on the cell line, VSe's functioned either to enhance NDV-mediated oncolysis and/or increase NDV replication in tumor cells. Taken together, these results suggest that transient vector- or VSe-mediated suppression of the host antiviral response allows for improved therapeutic transgene expression from oncolytic NDV.

Funding: OVC

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11) A systematic analysis of the functional utility of bone marrow-derived dendritic cells as a vaccine: Comparing several common culturing protocols

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Dendritic cell (DC) vaccines are emerging as a valuable biotherapeutic tool for treating cancers. However, culturing methods for DCs in preclinical studies vary dramatically in the literature. Here we systematically compare the functional utility of DCs differentiated from murine bone marrow (BM) using a variety of protocols. C57BL/6 mouse-derived DCs were differentiated from BM *in vitro* in the presence of GM-CSF on day 0, with or without the addition of IL-4 on Days 0-5. The DCs were stimulated with lipopolysaccharide (LPS) on day 7. The phenotype (maturation markers) and function (cytokine production) of DCs were characterized at several time points (12, 24, 36, 48 hours) post-stimulation via flow cytometry. Also, the primary vaccine potential of SIINFEKL peptide-pulsed, LPS-stimulated DCs was assessed, as was their function in a mixed lymphocyte reaction with allogeneic splenocytes. The introduction of IL-4 into a BM-derived DC culture is critical for developing an optimal DC cancer vaccine. Delaying the addition of IL-4 seemed to confer benefits in terms of the overall functionality of the DC cultures. Maturation-associated surface markers and cytokine production kinetics varied across cultures providing potential explanations for the functional differences across groups.

Funding: Terry Fox Research Institute and Art Rouse Cancer Biology Graduate Stipend

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12) Determinants of Tissue Specificity of the Small Ruminant Oncogenic Retroviruses, JSRV and ENTV

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Studying the oncogenic and pathogenic properties of retroviruses has helped shed light on mechanisms of replication, transcription and oncogenesis. Two small ruminant betaretroviruses which have contributed to our knowledge of oncogenesis are Jaagsiekte Sheep Retrovirus (JSRV) and Enzootic Nasal Tumour Virus (ENTV). JSRV and ENTV share high homology at the nucleotide and protein level, utilize the same cellular receptor, Hyal2, and drive oncogenesis through an envelope (Env) glycoprotein; yet, they target different areas of the respiratory tract, the lung and nasal tract, respectively. Interestingly, the morphology of JSRV Env-induced ovine lung adenocarcinoma is reminiscent of human bronchioloalveolar adenocarcinoma, a rare cancer largely affecting never-smoker females. This tissue selectivity and disease morphology make them great tools to study oncogenesis. To uncover the genetic determinant for tropism, we constructed JSRV-ENTV hybrids within a JSRV backbone and established different lentivirus pseudotypes. We also developed a tissue slice *ex vivo* model to circumvent JSRV and ENTV's cell polarity problems. Through infection of lung and nasal turbinate slices, we observed that the viral envelope protein is crucial for entry, but that the promoters of these viruses are likely responsible for tissue-specificity. Interestingly, we also found that Hyal2 abundance seems to act as the limiting step for ENTV entry. Furthermore, we show positive staining for ENTV Env in ENTV-infected ovine nasal turbinate tissue's extracellular matrix (naturally enriched with Hyal2). Our work points to a tentative novel ENTV oncogenic mechanism. Finally, our study also shows JSRV and ENTV tropism likely stems from a concerted effort involving the cell receptor, envelope protein, and promoters.

Funding: OVC PhD Scholarship, NSERC PSD, NSERC PDR

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13) Combining decitabine with oncolytic viruses to kill acute leukemias by oxidative stress

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Acute leukemias are aggressive hematological cancers. In general, T cell acute lymphocytic leukemias (T-ALL) and acute myeloid leukemias (AML) are difficult to treat, responses are limited, and current intensive chemotherapeutic regimens have a devastating impact on quality of life. Individuals who do respond to therapy often relapse with resistance to previously effective therapies.Oncolytic viruses (OV) are viruses that preferentially kill cancer cells. Decitabine is a DNA methyltransferase inhibitor that can mediate epigenetic changes with some efficacy in treating leukemias. While neither OVs or decitabine sufficiently control acute leukemias *in vivo*, we have found that pre-treatment with decitabine followed three days later with vesicular stomatitis virus (VSV) or Newcastle disease virus (NDV) leads to long-term remissions in mice using the EL-4 model of T-ALL, and the C1498 model of AML. Animals surviving long-term after combination therapy are also able to resist re-challenge with the same leukemia cells, indicating this therapy establishes immunological memory that could protect against a relapse. To assess the role of oxidative stress, N-acetyl cysteine, a reactive oxygen species (ROS) scavenger, was used *in vitro* and *in vivo*, which substantially reduced the efficacy of this combination therapy. In conclusion, combining decitabine with oncolytic virotherapy appears to be an effective treatment for acute leukemias, with the dominant mechanism of action being the induction of lethal oxidative stress.

Funding: Terry Fox Research Institute; Megan held an OVC Studentship and Ontario Graduate Scholarship.

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14) Plasma microRNA profile in pre- and post-amputation samples from canine appendicular osteosarcoma patients.

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Osteosarcoma is the most common primary bone tumor in both humans and canines. The median survival time of canine appendicular osteosarcoma is less than a year but clinical outcome is hard to predict between patients. The standard of care for appendicular canine osteosarcoma involves amputation of the afflicted limb and adjuvant chemotherapy. This treatment is aggressive and currently there is no decisive method to determine which dogs will benefit the most from this treatment. Therefore, it is necessary to discover and validate biomarkers that can predict clinical outcome after standard of care treatment of canine osteosarcoma. MicroRNAs are small non-coding RNAs that are involved in numerous cell processes and have potential as biomarkers of various diseases including cancer. MicroRNAs are found in various tissues and fluids, including plasma, providing easy collection by routine blood sampling. This study aims to profile the plasma microRNA expression in dogs with osteosarcoma, both before and after amputation as well as healthy dogs. By examining both pre- and post-amputation samples from the same dogs we hope to determine which circulating microRNAs are associated with the primary tumor. Pooled plasma samples of five dogs for each group were collected and the RNA was extracted, followed by a reverse transcription PCR of the RNA. Quantitative real-time PCR was conducted to determine miRNA expression using a QIAGEN canine miRNome array featuring 277 canine microRNAs. The microRNAs of interest from

these findings will be included in a custom microRNA array and examined with individual dog plasma samples.

Funding: Pet Trust, OVC MSc Scholarship, Graduate Excellence Entrance Scholarship

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15) Minimal residual disease detection in canine lymphoma

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Multicentric B cell lymphoma (mBCL) is the most common type of lymphoma in dogs with highly variable prognosis. Minimal residual disease (MRD) assessment by next generation sequencing (NGS) is used to monitor treatment efficacy and to detect early relapse in human medicine. The aims of this ongoing study are: 1) to determine whether MRD assessments in canine lymphoma can predict time to relapse or overall survival, 2) to determine the best sampling time points, and 3) to assess whether cell-free DNA (cfDNA) or cell-associated DNA (caDNA) collected from peripheral blood is a more sensitive sample type. To date, clinical inclusion criteria were met and study consent was obtained for 45 patients. Of these, 31 were of B cell lineage, and in 26 the neoplastic clone was detected in lymph node aspirates. Blood samples were collected at 2-4 week intervals during 26 weeks of treatment and at 1-3 month intervals thereafter, and separated into cell-associated (PBMC) and cell-free (plasma) fractions. For one patient MRD assessment by next generation sequencing (NGS) has been completed. This patient went into complete remission, relapsed 8 months after initiation of therapy and was euthanized 2 months thereafter. Using NGS, the neoplastic clone could be identified 7 weeks before clinical relapse. The neoplastic clone could be detected more readily from cfDNA (plasma) than from caDNA (PBMCs). Assessment of other patients is in progress, but preliminary data suggest that NGS might be more sensitive than clinical assessment for detecting relapse. Additional data are needed to substantiate these results.

Funding: OVC Pet Trust

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16) Rapid Prototyping of Patient-Specific Titanium Plates for Canine Cranioplasty

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Osteosarcoma and chondrosarcoma are two common tumours affecting the skull, in both humans and canines. Resecting these tumours produces cosmetic defects and leaves the underlying brain both exposed to external trauma and susceptible to infection from communication with sinuses. Canine cranioplasty involves replacing resected bone with a titanium mesh. Rapid prototyping titanium-alloy plates for cranioplasty presents a cost effective alternative while reducing time in the operating room. Diagnostic imaging techniques such as computed tomography (CT) can create patient-specific, virtual, three-dimensional (3D) anatomical structures. Patient's from Ontario Veterinary College CT images were evaluated using Osirix by board-certified radiologist and surgeon to determine the extent of the tumour and 5mm surgical margins. This CT data was exported as a DICOM file to a 3D imaging

program provided by Renishaw. A marginal defect was created using the 3D imaging software and a patient-specific titanium implant was created to cover this defect and exported as an STL file to Renishaw for printing. Evaluation of the CT scan by radiologist and surgeon was 10-15 minutes. Making a surgical plate in the Renishaw program was about 1 hour provided some expertise handling 3D software. Manufacturing of the titanium plate by Renishaw would take about 6-7 hours on average. Provided access to the technology, patient-specific 3D printed titanium implants can be manufactured for patient use within a one-week turnaround from diagnosis to planned surgery for canine patients undergoing cranioplasty.

Funding: OVC Pet Trust, Sheridan College, Renishaw, ADEISS

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17) Investigating molecular prognostic markers in canine mast cell tumours using tissue microarrays

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Mast cell tumors (MCTs) are the most common skin tumour of the dog, representing approximately 21% of all cutaneous tumours. The ability to accurately predict a tumour's behaviour is critical in directing patient therapy, and is especially important in canine MCTs, which range from benign to a fatal systemic disease. Although histological grading is useful for prognostication, it cannot predict the behaviour of each MCT. We hypothesized that biomarker expression in tumour cells will correlate with patient outcome, which will help guide future therapy. To this end, a pilot tissue microarray (TMA) from 40 cutaneous and subcutaneous canine MCTs has been created. A TMA is an array-based technique that enables high-throughput expression profiling of large numbers of tissues or tumours. We performed immunohistochemistry (IHC) for 10 different proteins (Beclin-1, C-CBL, VEGFR2, PDGFR-β, c-kit, mast cell tryptase, Ki-67, PCNA, vWF, CD31) on the MCTs, as well as positive and negative control tissue. Each tumour was analyzed to derive an H-score, which is a function of the intensity of IHC staining and the percentage of immunopositive cells. Results of a logic regression analysis of tumour expression of biomarkers such as Beclin-1 (a protein important in autophagy) and C-CBL (a ubiquitin ligase), among others, will be discussed. Beclin-1 and C-CBL are potentially promising new molecular biomarkers for canine MCTs that may improve prognostication and help guide the choice of adjuvant therapy.

Funding: OVC Pet Trust

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18) Effect of Rapamycin on Canine Mast Cell Tumor Cell Survival Following Radiation Therapy

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Mast cell tumor (MCT) is the most common cutaneous cancer in dogs. Surgery is the primary method of local treatment, however it isn't always possible to obtain adequate local control depending on the location of the tumour or the health of the dog. Radiation therapy is often used as a sole treatment or as an adjuvant for local control. Rapamycin is an inhibitor of mTOR, a pathway that is dysregulated

in several cancers. This pathway mediates cell growth, proliferation and survival. mTOR inhibition may also interfere with repair of DNA damage caused by radiation therapy. Literature has shown that rapamycin reduces cancer cell survival following radiation therapy. We therefore evaluated the effect of rapamycin in combination with radiation therapy on a canine MCT cell line: MCT-1. Cells were treated with rapamycin at three clinically achievable doses: 5.5nM, 11nM and 16.5nM for 24h or 48h, and clonogenic survival assays were performed. The experiments were repeated, with the addition of a single exposure of radiation therapy: 0, 3, 6 or 10 Gy. Rapamycin alone did not impact survival of MCT-1 cells. Radiation therapy dose positively correlated with MCT-1 cell death but neither rapamycin dose nor pre-treatment incubation period had any statistically significant effect on cell survival following radiation. A synergistic or additive effect of rapamycin with radiation therapy was not found, perhaps because MCT-1 cells are very sensitive to radiation therapy alone. Therefore, rapamycin combined with radiation therapy could have a more pronounced effect on more radio-resistant cancer cells.

Funding: OVC Pet Trust

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19) ICCI comparative oncology program: Clinical research studies in companion animal patients with cancer as models for human cancers

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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. Oncology-related clinical research trials at OVC HSC are performed with the Institute for Comparative Cancer Investigation (ICCI). Currently there are 12 studies recruiting oncology patients: 10 canine, 1 feline and 1 both species (http://ovc.uoguelph.ca/icci/trials). The ICCI is the only Canadian member in the National Institute of Health-National Cancer Institute (NIH-NCI) Comparative Oncology Trials Consortium (COTC). Many CA cancers share similar characteristics to human cancer types. Studies in CA cancer patients offer potential to fill the gap that exists between preclinical and phase I/II human studies. They also may benefit OVC veterinary patients e.g. novel techniques and treatment options.
The ICCI recently started participating in an early phase multi-centre trial with the NIH-NCI-COTC to evaluate a recombinant, attenuated *Listeria monocytogenes* expressing a chimeric human HER2/neu protein, following standard of care therapy, in dogs with osteosarcoma. A Phase I study which treated dogs with this vaccine demonstrated overall survival rates of **78%, 61% and 50%** at **1, 2 and 3 years**, respectively, compared to 1-2year survival rate of 15% with current standard of care (amputation and chemotherapy) (Mason et al., 2016). As this vaccine is not available anywhere else in Canada, the ICCI comparative oncology program has the potential to not only improve the lives of CA but also provide preclinical data for translational research relevant to human cancer, in particular pediatric osteosarcoma.

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

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20) The ICCI Companion Animal Tumour Sample Bank: facilitating translational cancer research

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The Companion Animal Tumour Sample Bank (CATSB) continues to successfully facilitate basic and translational veterinary oncology research. Currently, CATSB has over 1200 cases banked and has contributed samples to 16 intramural and extramural research projects. Located in the OVC HSC Mona Campbell Centre for Animal Cancer, 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 (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. The three most prevalent canine tumour types are soft tissue sarcoma, osteosarcoma, and mast cell tumours, but a variety of other neoplasms have also been banked. There are also currently 12 primary cell lines from canine and feline tumours available, with more in development. Researchers access samples by filling out a short application form. Prospective sampling can also be tailored to suit the needs of researchers. A cost-recovery fee (which is subsidized for University of Guelph researchers) is applied to enable the CATSB to continue its mission. In addition to samples, researchers can receive patient signalment, histopathology, and follow-up data. The ICCI continues to facilitate veterinary research to improve the lives of companion animals with cancer, with the potential to contribute to comparative human cancer research.

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

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21) RNA Disruption Indicates CHOP Therapy Efficacy in Canine Lymphoma

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Canine lymphoma is treated with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy protocol with remission rates of 73-92% with average first remission duration of 8-12 months assessed by physical measurement of lymph nodes. Prognostic indicators that correlate with length of or inability to achieve remission would be helpful. In a human breast cancer clinical trial, RNA integrity was identified as a useful prognostic marker. Therefore, the objective of this study was to investigate RNA disruption as a predictor of remission rate and duration in canine lymphoma treated with CHOP. Dogs diagnosed with lymphoma were treated with CHOP therapy. FNA (fine needle aspirate) samples were obtained at weeks 0 (pretreatment), 3, 6, and 11. Aspirates expressed into vials containing RNAprotect Cell Reagent (Qiagen) were stored until RNA isolation using miRNeasy kits™ (Qiagen, Inc). Aliquots were loaded onto RNA Nanochip™ (Caliper Technologies) and component RNAs resolved by capillary electrophoresis (Agilent 2100 Bioanalyzer). The

resulting electropherograms were analyzed using proprietary algorithms to quantify RNA concentration and disruption [RNA disruption index (RDI)]. In 41 dogs with lymphoma RNA concentration decreased in relation to clinical response. RDI values were higher in dogs that responded to treatment compared to non-responders (p=0.02). RDI values increased 0.13 times faster in B-cell lymphoma than T-cell lymphoma (p=0.02). The assessment of RNA disruption is a tool that could contribute to improved treatment and prognoses for dogs with lymphoma. Predicting risk of early relapse during chemotherapy could benefit patients by reducing ineffective treatment and facilitating earlier switch to more effective therapy.

Funding: OVC Pet Trust and RNA Diagnostics; and was assisted by the Oncology Service at OVC.

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22) Analysis of SNARE Regulation During Tumor Cell Invasion

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Tumor cell invasion involves targeted localization of proteins required for interactions with the extracellular matrix for proteolysis. The localization of many proteins during these cell-extracellular matrix interactions relies on membrane trafficking mediated in part by SNAREs. Previous research in our lab has found that SNARE proteins are required for the formation of invadopodia, which are F-actin rich structures that are able to degrade the surrounding ECM. The most recent work from our laboratory has provided insight as to how SNARE function is regulated during tumor cell invasion. Specifically, we have determined that the interaction of the SNARE, Syntaxin4, and its regulator, Munc18c is crucial to regulating trafficking of EGFR and MT1-MMP during invasion in MDA-MB-231 breast cancer cells. Disruption of this interaction between Stx4 and Munc18c perturbs the ability of Syntaxin4 to form complexes with its cognate SNARE partners, impairs trafficking of MT1-MMP and inhibits breast tumor cell invasion *in vitro.* Another known SNARE regulator is Cdc42, which regulates the vesicle SNARE, VAMP2. Previous research has shown that Cdc42 interacts with residues 1-28 in VAMP2. In order to inhibit the interaction between Cdc42 and VAMP2, residues 1-28 of VAMP2 are overexpressed in MDA-MB-231 cells. The effect that this regulation has on invadopodium formation and ECM invasion is being investigated, which indicate regulation of VAMP2 is required for tumor cell invasion. Our findings implicate Munc18c and Cdc42 as regulators of SNARE-mediated trafficking, advancing our understanding of the role of SNARE function in the localization of proteins that drive tumor cell invasion.

Funding: CRS and NSERC

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23) Studies of the regulation of dipeptidyl peptidase IV (DPPIV) expression and activity on colorectal carcinoma cells by J-series prostaglandins

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Dipeptidyl peptidase IV (DPPIV) is a multifunctional transmembrane protease on the surface of most human cells. Upregulated DPPIV activity may inhibit cancer progression by interacting with components of the tumour microenvironment. DPPIV facilitates cell adhesion to the extracellular

matrix, and associates with adenosine deaminase to reduce the accumulation of the immunosuppressant metabolite adenosine. DPPIV also degrades many signaling molecules through its dipeptidase activity, including the pro-tumorigenic chemokine CXCL12. Previous studies have shown that CXCL12's cell-surface receptor, CXCR4, exhibits an inverse relationship with DPPIV; such that increased DPPIV is accompanied by a concomitant decrease in CXCR4, and vice versa. CXCR4 cell-surface expression can be downregulated by J-series prostaglandins. Therefore, we investigated whether the J-series prostaglandins can also upregulate DPPIV expression and activity on colon carcinoma cells. We found that PGJ_2 and 15d-PGJ_2 both upregulate DPPIV dipeptidase activity at high concentrations when controlling for their cytotoxic effects. However, these prostaglandins did not appear to increase DPPIV gene or whole-cell protein expression. Upregulating DPPIV dipeptidase activity may inhibit the metastatic potential of colorectal cancer through increased degradation and inactivation of CXCL12.

Funding Source: NSERC

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24) The Effects of Nidogen-1 on Proliferation and Migration in Claudin-low Mammary Tumor Cells

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Breast cancer is the most common type of cancer among women, with one subset of the triple-negative subtype, claudin-low, known to be very aggressive and metastatic. For invasion and metastasis to occur, cancer cells must cross basement membranes, which contain structural proteins such as laminin and collagen IV and linking proteins such as perlecan and nidogen, and colonize on distant basement membranes. Nidogen is a glycoprotein that makes up 2-3% of basement membranes and has two types: nidogen-1 (NID1) and nidogen-2 (NID2). There have been limited studies on NID1 and cancer, with results demonstrating decreased invasiveness and metastatic capabilities in *Nid1* silenced cells of various cancer types. Through previous work, a murine cell line representative of the claudin-low subtype, known as RJ423, was developed; it demonstrated a 5000-fold increase in *Nid1* expression compared to the luminal subtypes. To test whether high *Nid1* expression contributes to the aggressive, metastatic nature of claudin-low tumors, *Nid1* levels were knocked down in RJ423 cells and proliferation and migratory capabilities were assessed. Immunofluorescence using a phospho-histone H3 antibody demonstrated that suppressing NID1 reduced RJ423 cell proliferation. Additionally, apoptosis was assessed using a cleaved caspase-3 antibody; however, cell death was not detected. Currently, migration is being evaluated using scratch wound and invasion assays. So far, a reduction in migration of the NID1 suppressed cells has been observed; however, further trials will be conducted to confirm results. Thus, this may provide a new area of NID1 targeted therapies to lessen the metastatic nature of claudin-low breast cancer.

Funding: CIHR and OVC Scholarship

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25) The use of polyacrylamide hydrogels to evaluate the impact of extracellular matrix stiffness on tumorigenic properties of canine osteosarcoma

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Osteosarcoma (OSA) is the most common primary bone tumor in canines with a poor prognosis due to metastasis. The physical and mechanical properties of the extracellular matrix (ECM) are altered during tumor progression, and may facilitate metastasis. Transcriptional co-activators TAZ/YAP transduce mechanical cues into cellular responses which involves cytoskeletal modifications. The protein ezrin links the cytoskeleton to the plasma membrane, and therefore may transduce external signals to alter cytoskeletal dynamics, and thus TAZ/YAP activity. TAZ, YAP, and ezrin have been independently implicated in human and canine OSA progression, but their connection as part of the same signalling cascade has not been previously demonstrated. This research aims to determine the potential link between ezrin and TAZ/YAP signaling and their role in OSA progression. To do this, collagen type I-coated polyacrylamide hydrogels with stiffnesses of 0.5, 5, 50, and 100 kPa were prepared and verified using atomic force microscopy. Canine OSA cell lines were seeded onto the hydrogels and phase contrast microscopy assessed cell morphology, while immunofluorescence evaluated ezrin activation, TAZ/YAP localization, and cytoskeletal changes. Cells on low stiffness hydrogels demonstrated a round morphology compared to those on stiffer hydrogels, which displayed elongation. Changes in ezrin activation and TAZ/YAP localization were also observed across stiffnesses. In the future, we aim to determine the functional consequences of this ezrin-TAZ/YAP signaling axis at different stages of progression by comparing primary and metastatic OSA cell lines. Results from this study will improve our knowledge of OSA biology and may lead to more effective therapeutic targets.

Funding: OVC Pet Trust and NSERC

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26) Overexpression of Nck Increases Invadopodia Formation and Invasion of Breast Cancer Cells

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Despite recent advances in the treatment of breast cancer metastatic breast cancer remains incurable, and the molecular signals that promote the invasion of cancer cells from primary tumours to secondary sites are poorly understood. During tumour cell invasion metastatic cancer cells form actin-based structures, called invadopodia, that protrude into, and degrade the extracellular matrix (ECM) that surrounds them. This process requires rearrangement of the actin cytoskeleton. Nck is an adaptor protein known to interact with proteins that rearrange the actin cytoskeleton and has been implicated in invadopodia formation and cancer cell invasion. Nck is also overexpressed in the more metastatic breast cancer subtypes. However, effect of overexpression of Nck *in vitro* is unknown. We have shown that Nck overexpression increases invadopodia formation, gelatin degradation and invasion of MDA-MB-231 cells, a human breast cancer cell line. This supports a role for Nck as an important signaling protein in invadopodia formation and invasion in breast cancer.

Funding: Canada Research Chairs Program

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27) The use of fibroblast spheroids as a model for assessing the ability of bacteriophages to penetrate tumour stromal tissues

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Solid tumours are characterized by a complex structure comprising extracellular matrix, neoplastic cells and stromal cells, each presenting particular barriers to conventional anticancer chemotherapy as well as carrier-mediated drug delivery. Poor penetration of therapeutics into the interstitial tumour microenvironment remains a problem, with drugs accumulating primarily in the regions of tumours that are situated closer to blood vessels. In this work, we used NIH-3T3 fibroblast multicellular spheroids to first reproduce the stroma of solid tumours and examine infiltration of bacteriophages, a novel means of delivering a therapeutic hit. Bacteriophages do not infect eukaryotic cells, yet they have been demonstrated to penetrate mucosal barriers, including multiple layers of epithelial cells and endothelium. Furthermore, bacteriophages accumulate in both the blood and various body organs. We have used fluorophore-conjugated bacteriophage lambda to visualize and assess phage infiltration. We posit that the unique capability of bacteriophages to traverse the tumour interstitium will be reproduced in fibroblast and carcinoma aggregates and that they will accumulate in the inner regions of spheroids, providing a model to assess modifications that will enhance infiltration. Preliminary results with NIH-3T3 spheroids point to localization of bacteriophage lambda occurring in spheroids as early as 6 hours of treatment. These results contribute to the possibility of the development of bacteriophages as nanocarriers for targeted delivery.

Funding Source: NSERC

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28) Using next generation sequencing to improve the sensitivity of molecular clonality assays for canine B cell proliferations.

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Differentiating B cell lymphoma from inflammation is important because it impacts the choice of treatment. Clonality testing can facilitate this distinction by assessing immunoglobulin heavy chain (IGH) gene diversity. Though several PCR-based assays have been developed in the past 20 years, their sensitivity in routine diagnostics continues to be suboptimal. This was thought to be due to the inability of the assays to detect some IGH gene variants. The current study aimed to improve the sensitivity of the existing assays by: a) characterizing the canine IGH repertoire using next-generation sequencing (NGS) and identifying frequently used genes to direct assay design, and b) modifying assays using NGS data to allow for more comprehensive detection of IGH. By sequencing IGH mRNA from 3 lymphatic organs from 3 healthy dogs each, over 2.5 million IGH sequences were obtained. The most frequently used 5' variable and 3' joining gene sequences across the IGH repertoire were identified, including IGHV4-1 (16.5%) and IGHJ6 (14.6%). Sequence alignments

between primers from 5 previously described B cell clonality assays and the NGS data set revealed that many assay primers sequences were poorly matched for frequently used sequences; modified primer sets incorporated additional primers to better detect IGH genes containing these sequences. The sensitivity of modified assays was up to 34.6% higher than the original assays. The improved assays will reduce the likelihood of misdiagnoses and hence result in better patient care and treatment. This study was also the first comparative assessment of canine B cell clonality assays.

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29) Optimization of an explant culture model to characterize cancer-associated exosomes in canine osteosarcoma

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Osteosarcoma is the most common bone tumor in canines and humans. Previous studies have shown that both tumor and tumor-associated cells promote osteosarcoma progression through extracellular vesicle secretion, such as exosomes. Various factors within the environment can impact exosome release. The goal of this research was to develop an explant culture model to purify and characterize extracellular vesicles from canine osteosarcoma tissue, focusing on exosomes. We reasoned that explant cultures would allow for a more accurate representation of tumor exosomes in *vivo*, enhancing the potential for clinical translation. With owner consent, tumor and normal bone (control) samples were obtained using sterile tools following limb amputation. Tissue samples were washed with PBS, mechanically dissociated, and incubated in culture media under standard conditions overnight. The next day, conditioned media was recovered, and fresh media was added to the explants and incubated for another 48 hours. Media from both time points were centrifuged to remove cell debris, and the supernatant was collected. qEV size exclusion chromatography columns were used to isolate extracellular vesicles from the explant media. Vesicles were characterized via immunoblotting, electron microscopy, and particle tracking. Both tumor and normal tissue media contained extracellular vesicles, expressed markers of exosomes, and displayed different elution profiles. Electron microscopy and particle tracking showed an enrichment of particles with morphology and size typical of exosomes. Further optimization steps are required to improve exosome yield and purity prior to mass spectrometry. This explant culture model provides a novel approach to study the role of exosomes in osteosarcoma.

Funding: OVC Pet Trust and NSERC

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30) Recovery and analysis of circulating tumour cells from the peripheral blood of stages III and IV colorectal and breast cancer patients

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Optimising future approaches for the personalized treatment of patients with metastatic cancers will benefit from our ability to sample cells emigrating from the tumour and detectable in peripheral

blood. Existing methods to isolate circulating tumour cells (CTCs) do not address cellular functions and rely heavily on chemical or physical proprties. In this study, we are using a technique based on the CTCs' ability to interact with extracellular matrix (ECM) proteins for a more functional capture. A group of 40 patients with Stage III or IV colorectal or breast cancers provided peripheral blood samples at the time of their clinical visit. The blood cells were fractionated by density centrifugation on Ficoll-Paque PLUS® and CTCs were recovered from the interface together with leukocytes. This cell population was washed and plated on substrata composed of selected ECM proteins including collagen, fibronectin, laminin and combinations. After 18h of incubation at 37°C, non-adherent cells (most leukocytes) were washed away and the remaining cells (primarily CTCs) were fixed with paraformaldehyde, followed by immunofluorescence staining for marker proteins. Data showed differences in recovery depending upon the ECM proteins used in the capture substratum, with CTCs staining positive for epithelial markers EpCAM and pan-cytokeratin and negative for leukocytic antigens. So far, a minimum of ~50% recovery of cancer cells has been documented in calibration experiments and further efforts are directed towards characterizing isolated CTCs for expression of chemokines. Tracking patients at 6-12 months after their initial visit allows for comparison of changes in marker expression and disease progression.

Funding: CIHR
