Program

ICCI Cancer Research Symposium

Wednesday May 13, 2009
Room 1714 LLC, Ontario Veterinary College
University of Guelph

Welcome and Introductory Remarks

Welcome to the second annual University of Guelph Institute for Comparative Cancer Investigation Cancer Research Symposium. The ICCI was established in 2007, with a mission to improve companion animal health, promote interdisciplinary cancer research, and train future generations of cancer care specialists and scientists. The Institute builds on the existing expertise here at the University of Guelph in both applied clinical care and basic cancer biology, to take advantage of the tremendous potential at Guelph to integrate cancer biology "bench to patient and back to bench".

Today's symposium brings together cancer investigators from all stages of their careers and multiple academic units, and we trust you will find their presentations both informative and inspiring. New this year is a poster presentation session that especially highlights the activity of research trainees. Thank you to everyone who submitted an abstract for today's symposium- we feel we have an exciting program with lots of opportunity for discussion and collaborative interaction. We welcome any comments or suggestions concerning the format and speakers for future years. Last, but not least, we would like to thank the OVC Dean's Office and the Arthur Willis Visiting Professorship for their financial support.

Co-Organizers
Brenda Coomber and Jon LaMarre.
Biomedical Sciences, University of Guelph

Welcome to the second annual Guelph ICCI Cancer Research Symposium. The results being communicated today reflect the complexity of cancer and the diverse ways in which we attempt to understand this disease from the early molecular events investigated in cultured cells to the clinical manifestations in various animal species bearing tumors that are either experimentally-induced or naturally-occurring. The diversity of research projects and the related findings are a clear reflection of the benefits derived from our unique university environment. Our cancer research is truly comparative and our basic and clinical research collaborations bear novel translational discoveries. Everyone benefits from this arrangement and these exceptional relationships, from the students we train to the patients we treat. We are clearly very fortunate.

Gordon M. Kirby DVM, MSc, PhD
Associate Dean, Research & Innovation
Professor, Department of Biomedical Sciences
Ontario Veterinary College

Schedule

May 13, 2009 Room 1714 OVC LLC

8:30 Welcome and Introductory Remarks
Gordon Kirby, Associate Dean, Research & Innovation, OVC
Kevin Hall, Vice President, Research
Brenda Coomber, Co-Director, ICCI

8:45 "A chemotherapy drug pump: progress and challenges"
Frances Sharom, Professor, Molecular and Cellular Biology, CBS

9:05 "Omega-3 fatty acids and breast cancer"
David Ma, Assistant Professor, Human Health & Nutritional Sciences, CBS

9:25 "Involvement of a novel vitamin D receptor in growth inhibition of breast cancer cells"
Cynthia Richard, Postdoctoral Fellow, Human Health & Nutritional Sciences, CBS

9:45 "Pathological fracture in dogs due to primary bone tumors: a Retrospective study"
Sarah Boston, Assistant Professor, Clinical Studies, OVC

10:05 "Understanding the needs and expectations of clients pursuing oncology treatment for their pet at a tertiary referral center"
Debbie Stoewen, PhD Candidate, Population Medicine, OVC

10:25 Coffee (Room 1707 B/C)

11:00 Keynote Presentation: "The Growing Role of Comparative Oncology in Cancer Research"
Dr. Barbara Kitchell, Arthur Willis Distinguished Speaker
Department of Small Animal Clinical Sciences, Center for Comparative Oncology, College of Veterinary Medicine, Michigan State University

12:15- 1:30 Lunch (on your own)

1:30 "Ovine pulmonary adenocarcinoma: a novel model for human lung cancer"
Sarah Wootton, Assistant Professor, Pathobiology, OVC

1:50 "Sub-molecular analysis of P53 cancer suppressor"
David K.Y. Chiu, Professor, Department of Computing and Information Science, CPES

2:10 "Mechanics of naked function in inhibiting Wnt signaling"
Terry Van Raay, Assistant Professor, Molecular and Cellular Biology, CBS

2:30 “Understanding the mechanisms of breast cancer metastasis”
Alicia Viloria-Petit, Assistant Professor, Biomedical Sciences, OVC

2:50 “The psychosocial impact of cancer, psychosocial oncology and intervention fidelity”
Michele Preyde, Associate Professor, Family Relations & Applied Nutrition, CSAHS

3:10 “Gastrointestinal stromal tumours: a new era in cancer chemotherapy and patient self-empowerment”
P. David Josephy, Professor, Molecular and Cellular Biology, CBS, and President, GIST Sarcoma Life Raft Group, Canada

3:30 Closing Remarks
Jonathan LaMarre, Professor, Biomedical Sciences

3:35-5:00 Poster session and reception (Room 1707 B/C; cash bar).

Guelph ICCI Cancer Research Symposium
Keynote Presentation

The Growing Role of Comparative Oncology in Cancer Research

Barbara Kitchell, D.V.M., Ph.D., DACVIM.
Professor of Oncology
Center for Comparative Oncology, Michigan State University

11:00 am, Wednesday May 13, 2009
1714 LLC, OVC

Abstract: Advances in our understanding of the molecular aspects of cancer present many opportunities for development of molecularly targeted therapeutics and the potential for individualized medicine. Robust animal models with tumor characteristics replicating those seen in human malignancies must be developed in order to evaluate such targeted therapeutic strategies. Companion dogs with spontaneously arising malignancies may augment traditional rodent models, so that disease biology and targeted therapeutics may be better evaluated in the translational setting.

Speaker Biography

Dr. Barbara E. Kitchell graduated from Purdue University School of Veterinary Medicine in 1979. Dr. Kitchell completed an internship at the University of Minnesota, then residency in Small Animal Medicine at UC Davis. She started an Oncology referral center at Special Veterinary Services, Berkeley, California in 1985. She received a Ph.D. degree (emphasis in Cancer Biology) from the Department of Comparative Pathology at UC Davis in 1994. In addition, Dr. Kitchell completed a postdoctoral fellowship in the Department of Comparative Medicine, Stanford Medical School from 1990-1994. She returned to academic medicine in 1994 as Assistant Professor in the Department of Veterinary Clinical Medicine, University of Illinois School of Veterinary Medicine. Dr. Kitchell joined the faculty of Michigan State University in 2004, where she is acting as Director of the Center for Comparative Oncology. Dr. Kitchell is an ACVIM Diplomate in the specialties of Internal Medicine and Oncology. She has received numerous awards including the National Cancer Institute Physician Scientist Award, the Dean's Postdoctoral Fellowship Award at Stanford, and the Gaines Cycle "Golden Fido" award for Veterinarian of the Year in 1993. She was a selected participant at 2 workshops (Molecular Biology of Cancer in 1993 and Methods in Clinical Cancer Research in 1997), sponsored by the American Association for Cancer Research and is an active member of that organization. She is currently president of the Veterinary Cancer Society, as member of the Veterinary Oncology Trials Consortium and the Comparative Canine Oncogenomics Consortium at the National Cancer Institute. Her areas of interest include medical oncology, molecularly targeted therapeutics, translational modeling in spontaneously arising tumors, mechanisms of drug resistance and cancer biology. She is the author of numerous scientific publications and chapters, and is an internationally recognized authority in comparative oncology.

Abstracts for Oral Presentations

A chemotherapy drug pump: progress and challenges.

Frances J. Sharom, Ronghua Liu, Richard G. Mather and Miguel R. Lugo. Department of Molecular and Cellular Biology, University of Guelph

Multidrug efflux pumps that are members of the ABC superfamily are known to play an important role in resistance of cancers to chemotherapy drugs. Our laboratory is studying the structure and function of P-glycoprotein (ABCB1), the best characterized of these pumps. Many chemical modulators are known to block the transport activity of P-glycoprotein, and their use in combination with chemotherapy drugs may improve the future success of clinical treatment.

P-glycoprotein is a 170 kDa integral protein comprising two cytoplasmic nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs). Transport is driven by hydrolysis of ATP, which promotes tight association of the NBDs to form a nucleotide sandwich dimer. In our laboratory, purified P-glycoprotein is isolated and studied in either detergent solution or reconstituted lipid vesicles, where it displays ATP-driven transport of structurally unrelated amphipathic drugs. We have developed fluorescence spectroscopic approaches to quantify the binding affinity of P-glycoprotein for nucleotides, drugs and modulators. We are also mapping the large, flexible substrate binding pocket, which can bind two drugs simultaneously. Drugs enter this site, which is contained within the TMDs, after partitioning into the membrane. A recently published high resolution crystal structure of P-glycoprotein has revealed the principles of multidrug binding.

Omega-3 Fatty Acids and Breast Cancer

David W.L. Ma, Department of Human Health and Nutritional Sciences, University of Guelph

Breast cancer is a leading cause of cancer death in Canadian women. Emerging evidence suggests that early life exposure to specific dietary factors such as marine oils, containing n-3 polyunsaturated fatty acids (PUFA), during critical periods of development may potentially influence cancer risk in adulthood. Genetic and dietary mouse models will be used to investigate the effect of n-3 PUFA on mammary gland development, and how n-3 PUFA influences cell signaling through membrane microdomains known as caveolae. It is anticipated that this research will enhance our fundamental knowledge of the mechanisms regulating mammary gland development and provide novel insight into therapeutic strategies for the prevention of breast cancer.

Involvement of a novel vitamin D receptor in growth inhibition of breast cancer cells.

Richard CL and Meckling KA, Department of Human Health and Nutritional Sciences, CBS, University of Guelph

In addition to well-recognized roles in calcium homeostasis and bone development, vitamin D inhibits the growth of several cancer types, including breast cancer. Although cellular effects of 1,25 dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] traditionally have been attributed to activation of a nuclear vitamin D receptor (VDR), a novel receptor for $1,25(OH)_2D_3$ called $1,25D_3$-MARRS (Membrane-associated, rapid response steroid-binding) protein was identified recently. We sought to determine the involvement of $1,25D_3$-MARRS in the growth-inhibitory activity of $1,25(OH)_2D_3$ in breast cancer cells. We stably transfected MCF-7 human breast cancer cells with a ribozyme construct designed to knock down $1,25D_3$-MARRS mRNA. MCF-7 clones in which $1,25D_3$-MARRS receptor levels were knocked down showed increased sensitivity to $1,25(OH)_2D_3$ (IC_{50} 56 +/- 24 nM) compared to control cells (319 +/-181 nM; $P<0.05$). Reduction in $1,25D_3$-MARRS receptor lengthened the doubling time in transfectants treated with $1,25(OH)_2D_3$. Knockdown of $1,25D_3$-MARRS receptor also increased the sensitivity of MCF-7 cells to agents which mimic vitamin D (KH1060 and MC903), but not to unrelated treatments (all-trans retinoic acid, serum starvation, or the isoflavone, pomiferin). These results suggest that the expression level $1,25D_3$-MARRS influences the growth inhibitory activity of $1,25(OH)_2D_3$ in breast cancer cells, pointing to its importance in determining cell fate.

Pathological fracture in dogs due to primary bone tumors: A retrospective study.

Sarah E. Boston, DVSc, Diplomate ACVS, Jitender Bhandal DVM, Clinical Studies, OVC, University of Guelph

The purpose of this retrospective study was to evaluate clinical presentation of pathological fractures associated with a primary bone tumor in dogs and to assess treatment and survival times of these patients. Medical records of dogs that presented with pathological fracture associated with a primary bone tumor between January, 1997 and May, 2008 were reviewed. Dogs were included in study if they had radiographic evidence of a pathologic fracture and a presumptive or definitive diagnosis of osteosarcoma. Twenty-five dogs were included in this study. Radiographic details, histopathology and/or cytology findings of the lesion were recorded. Overall median survival time and median survival time of the treated dogs were calculated. The age, sex, breed and other concurrent treatment were also evaluated. A majority of the dogs experienced minor trauma and 60% of the dogs exhibited lameness preceding the fracture. Rottweilers, followed by Irish wolfhounds and Greyhounds were the most common breeds that presented. Most commonly, the fractures were nondisplaced with minimal comminution. None of the dogs had radiographic evidence of pulmonary metastases at the time of presentation. Immediate and delayed euthanasia were performed in 52% and 16% of the cases respectively. One case was not treated and died at home 90 days after diagnosis (4%). Three patients were treated with amputation alone, one with amputation and chemotherapy, and three with internal fixation using an interlocking nail. Overall median survival time was 110.5 days with a range of 0-613 days. Median survival time of the treated patients was 406.5 days. Histologic confirmation of osteosarcoma was available in all of the treated patients and in 6 cases that were euthanized via post-mortem or bone biopsy. Treatment for pathologic fracture due to a presumptive osteosarcoma may be considered. Options available to the owner for treatment include amputation and internal fixation, with and without adjunctive chemotherapy.

Understanding the Needs and Expectations of Clients Pursuing Oncology Treatment for their Pet at a Tertiary Referral Center

Stoewen DL, Coe JB, Dewey C, MacMartin C. Ontario Veterinary College and College of Social and Applied Human Sciences, University of Guelph.

Research in veterinary medicine has identified that veterinarians' perceptions of clients' needs and expectations are not always consistent with clients' actual needs or expectations. Although a number of published studies have examined the needs and expectations of veterinary clients within everyday companion animal practice, none have explored the needs and expectations specific to the unique challenges within cancer care practice. Veterinary medicine offers sophisticated technology for cancer; however, knowledge of the complexities of client care within this context to complement this highly specialized service is currently lacking. Evidence suggests that the attachment between people and their pets is greater than ever, and that a heightened human-animal bond translates into increased owner commitment to their animal. The occurrence of cancer in dogs and cats has also steadily been on the rise and is expected to continue as veterinary medicine finds ways to further improve patient health and in turn extend the life expectancy of companion animals. Therefore, together a heightened human-animal bond and increased occurrence of cancer in the pet population are likely to amplify the demands around the cancer treatment of companion animals, challenging veterinarians from both medical and social perspectives. Understanding the needs and expectations of owners of cancer care pets is important. It follows the standard of bond-centered practice. Within the emotionally charged context of cancer treatment, a greater awareness of and response to client needs and expectations will better enable the cancer treatment team to support both clients and patients to maximize the potential for good outcomes in this unique area of veterinary care.

The Growing Role of Comparative Oncology in Cancer Research

Barbara Kitchell, Michigan State University

Advances in our understanding of the molecular aspects of cancer present many opportunities for development of molecularly targeted therapeutics and the potential for individualized medicine. Robust animal models with tumor characteristics replicating those seen in human malignancies must be developed in order to evaluate such targeted therapeutic strategies. Companion dogs with spontaneously arising malignancies may augment traditional rodent models, so that disease biology and targeted therapeutics may be better evaluated in the translational setting.

Ovine pulmonary adenocarcinoma: a novel model for human lung cancer

Sarah Wootton, Department of Pathobiology, OVC, University of Guelph

Research in our laboratory is largely directed at understanding the molecular pathogenesis of virally induced cancers, specifically that of retroviruses. A major focus of research currently under investigation involves the study of Jaagsiekte sheep retrovirus (JSRV), the causative agent of a transmissible form of lung cancer in sheep known as ovine pulmonary adenocarcinoma (OPA). OPA is of particular interest to us due to striking clinical and histological similarities with human lung adenocarcinoma, particularly bronchioloalveolar carcinoma (BAC) - the most common type of lung cancer among non-smokers. JSRV is an acutely transforming replication-competent retrovirus that does not harbour any known oncogenes yet it can induce tumors in as little as two weeks in newborn sheep. Intranasal administration of immunodeficient mice with a replication-defective adeno-associated virus vector (AAV) expressing the JSRV envelope protein (ARJenv) leads to the development of multifocal lung cancer that progresses from adenoma to adenocarcinoma thereby demonstrating that the envelope protein (Jenv) is necessary and sufficient to induce tumors *in vivo* and can do so in a receptor-independent manner. Tumors are comprised exclusively of alveolar type II (ATII) cells suggesting that cells of this lineage are primary targets for Jenv-mediated oncogenesis and that susceptibility may be cell type specific. One model to explain these findings is that Jenv is transforming cells that are already "primed" in some way to evade apoptosis and proliferate indefinitely - properties inherent to stem cells. The hypothesis that Jenv is specifically transforming regional stem cells in the distal lung is partially supported by the fact that of a population of stem cells located in the distal lung of mice, called bronchioalveolar stem cells (BASCs), are thought to be the cells-of-origin of lung adenocarcinoma. Our current hypothesis is that Jenv is specifically transforming BASCs and experiments are underway to address this exciting possibility as well as to elucidate the signalling pathways involved in the development of this virally-induced lung tumor in both sheep and mice.

Sub-molecular Analysis of P53 Cancer Suppressor

David K.Y. Chiu, Thomas Lui, Yan Wang, Department of Computing and Information Science, University of Guelph

It is not usual to think of computing as an important scientific method when the data are sparse and the pattern is simple. With the enormous growth of scientific data in databases, computing has rediscovered its strengths as an important paradigm in studying problems in data-rich biological sciences. Consequently, powerful algorithms that can synthesize molecular patterns have enormous advantage. This talk will introduce methodologies that synthesize statistical patterns conveying different types of inherent association information from aligned protein family. The idea is that by using statistical patterns that deviate from the expected observations of a predefined prior model, a synthetic method may identify new information that may not be previously known. I will discuss methods that make inferences to important sites in the 3-dimensional structure and other functionality from families of aligned sequences. P53 protein is a transcription factor found in multicellular organisms that regulates the cell cycle. In humans, it is found to involve in the prevention of cancer because of its relation to genome mutations. It was voted molecule of the year by Science magazine and is known as the "the guardian of the genome" due to this role in conserving genome stability. Even though its 3-dimensional structure and the central DNA-binding core domain is known, further analysis of this region is necessary in understanding its anti-cancer mechanisms. We have identified regions that have consistent statistical properties in eight sub-exon regions, as well as four sub-regions (called D-regions) in the binding domain that are all in the surface of the molecule. These four D-regions if not mutated, rarely (negatively) associated with cancer. The mutation characteristics of the regions identified also are associated with hereditary factors in different types of cancer as compared to human twin study.

Mechanics of Naked Function in inhibiting Wnt Signaling

Terry Van Raay[1], Haiting Ma[2], Nick Fortino[1], Lila Solnica-Krezel[2] and Bob Coffey[3]
[1]Molecular and Cellular Biology, University of Guelph; [2]Biological Sciences, Vanderbilt University;
[3]Dept of Medicine and Cell and Developmental Biology, Vanderbilt University Medical Center

Recently, it has been suggested that cancer is a stem cell disease. Further, evidence has shown that many cancers, colon cancer in particular, are caused by mutations in genes that belong to one particular cell signaling pathway; the Wnt pathway. These mutations result in uncontrolled proliferation of cells leading to cancer. The objective of my research is to understand how the Wnt signaling pathway controls its own activity using an in vivo stem cell model. To this end I am taking advantage of zebrafish. Using blastula stage embryos, which contain only multipotent stem cells, I am able to stimulate Wnt signaling in virtually all of these cells. Currently, my research, and the focus of this seminar, is to determine exactly how a Wnt induced negative feedback regulator, Naked, functions to inhibit Wnt signaling.

Understanding the mechanisms of breast cancer metastasis

Alicia Viloria-Petit, Assistant Professor, Department of Biomedical Sciences, OVC, University of Guelph

Breast cancer is the most common malignancy in women in North America. Nearly 90% of breast cancer mortality is caused by metastatic spread of the tumor to distant organs. Research in our laboratory is focused at understanding the molecular and cellular processes involved in breast cancer progression to an invasive, metastatic disease. To this end, we use both three-dimensional culture models, which mimic breast tissue organization and function in an appropriate extra-cellular microenvironment, as well as mouse models of metastatic mammary cancer. We are additionally interested in translating the findings we obtain with our tumor models to more clinically oriented studies. For this purpose, we look at the expression and/or activation status of candidate molecules with potential roles in metastatic spread and correlate them with tumor parameters of current use in predicting patient prognosis and response to therapy. Our ultimate goal is to improve our understanding of the molecular basis of breast cancer progression and consequently, the clinical management of the disease.

The Psychosocial Impact of Cancer, Psychosocial Oncology and Intervention Fidelity

Michèle Preyde, Associate Professor, Family Relations & Applied Nutrition, CSAHS, University of Guelph

Cancer is a major health issue that affects a significant proportion of the population. In Canada, current incidence estimates indicate that approximately 39% of women and 44% of men will develop cancer in their lifetime (Canadian Cancer Society). Considerable research indicates that many oncology patients and their families experience critical levels of distress. Psychosocial oncology interventions have been developed to target the source of stress and may be essential for helping patients and families cope and improve their quality of life. We have explored patients' experiences at a Regional Cancer Centre. Patients rated the medical and allied health professionals and the psychoeducational supports quite favourably; however, they also highlighted areas of personal concern. We have also examined the effectiveness of psychosocial oncology interventions; however, study results were inconsistent. We have also assessed the degree of integrity to intervention modals. Intervention fidelity refers to strategies employed to ensure that the intervention is implemented and delivered as it was intended. We conducted a systematic review of fidelity on psychosocial oncology interventions. It was estimated that adherence to fidelity measures was moderate. These results have implications for evidence-based practice and knowledge translation.

Gastrointestinal stromal tumours: a new era in cancer chemotherapy and patient self-empowerment.

P. David Josephy, Department of Molecular and Cellular Biology, University of Guelph, and GIST Sarcoma Life Raft Group Canada

Gastrointestinal stromal tumour (GIST) is a soft tissue sarcoma of the gastrointestinal tract, most commonly occurring in the muscular wall of the stomach. GISTs arise in the interstitial cells of Cajal, the pacemaker cells controlling GI peristalsis. GIST cells usually carry an activating mutation in either the c-kit (CD117) tyrosine kinase or PDGFR (platelet-derived growth factor receptor) alpha genes. Targeted drug therapy with inhibitors of c-kit, such as glivec (imatinib mesylate, introduced c. 2000) and sutent (sunitinib, introduced c. 2006) has revolutionized the treatment of metastatic GIST. Recently, glivec has been shown to be highly effective in the adjuvant setting, as well. These developments have been hailed as among the greatest breakthroughs in the history of cancer chemotherapy. GIST is a rare disease (annual incidence about 1 per 100,000 population). Before the advent of glivec therapy, GIST patients were isolated and disempowered, and the disease was almost ignored by the mainstream cancer research and patient support organizations. Simultaneously with the recent pharmacological advances, and facilitated by the growth of internet communications, GIST patients and caregivers have organized several non-profit support groups dedicated to pushing the science and medicine forward as rapidly as possible. I will discuss both of these paradigm-shifting changes: the science of GIST targeted therapy, and the emerging concept of activist patient/ caregiver participation in the medical research enterprise.

http://www.liferaftgroup.ca/
http://www.liferaftgroup.org

Abstracts for Poster Presentations

#1) Functional autocrine and intracrine VEGF/VEGFR2 signaling loop in malignant melanoma: impact on tumor progression

Adamcic U and Coomber BL, Biomedical Sciences, OVC, University of Guelph

Vascular endothelial growth factor-A, or VEGF, produced by tumor cells promotes angiogenesis by binding and activating two tyrosine kinase receptors, VEGFR-1 and VEGFR-2 on endothelial cells. Recent data suggests that VEGF can directly aid in tumor progression by binding and activating VEGFR2 expressed by different types of cancer cells, including malignant melanoma. This autocrine VEGF/VEGFR2 loop stimulates survival of melanoma cancer cells by activation of PI3-kinase pathway. Here we report the expression of VEGFR2 at the protein and mRNA level by human melanoma cells. When compared to endothelial cells, the expression of VEGFR2 receptor was significantly lower in both primary and metastatic melanoma cells. VEGF produced by melanoma cells *in vitro* was able to phosphorylate VEGFR2 receptor in both primary and metastatic cells. However, metastatic melanoma cells produced approximately 13 fold greater amounts of VEGF compared to the primary melanoma cells and had concomitant enhanced levels of phosphorylated VEGFR2. Through western blotting of nuclear and cytosolic protein lysates, we also detected the presence of an activated intracrine VEGF/VEGFR2 loop in primary and metastatic melanoma cells. In addition, the significant increase in VEGF production by metastatic melanoma compared to primary melanoma correlates with a significant decrease in the production of the endogenous anti-angiogenic factor thrombospondin-1 (TSP-1) by metastatic melanoma compared to primary melanoma cells. Taken together, our results support a direct role for VEGF on malignant melanoma cells, and suggest that anti-cancer therapies targeting the VEGF/VEGFR system may act differentially depending on progression of this disease. Funded by the CIHR.

#2) Blockade of VEGFR2/KDR increases malignancy in human epithelial ovarian carcinoma

Sirin A. Adham, Ifat Sher, and Brenda L. Coomber, Biomedical Sciences, OVC, University of Guelph

Human epithelial ovarian carcinoma (EOC) is the most lethal neoplasm affecting the female genital tract. Vascular Endothelial Growth Factor (VEGF) and its tyrosine kinase receptor KDR (VEGFR2) are overexpressed in these lesions. We previously demonstrated that this signaling loop provides a survival pathway for human EOC growth in suspension, similar to what would occur in ascites fluid that accumulates in the peritoneal cavity of the patient. Chemoresistance is a major problem for successful therapy of this cancer, however, some progress has recently been made using bevacizumab (Avastin; a monoclonal antibody against VEGF) in adjuvant settings. We therefore explored whether a more complete blockade of VEGF signaling would be an effective strategy for cancer control by knocking down KDR expression in chemoresistant OVCAR-3 EOC cells using siRNA. Cells with KDR knockdown demonstrated more aggressive subcutaneous growth in vivo. In addition, when cells lacking KDR were implanted into the peritoneal cavity of immune deficient mice, these cells enhanced the accumulation of ascites characterized by higher VEGF levels. The cells lacking KDR showed increased Neuropilin-1 expression and decreased expression of some adhesion proteins, notable cadherins and integrins. Evaluation of 80 clinical cases of EOC for NRP-1 versus KDR expression showed a significantly higher NRP-1:KDR ratio with cancer progression. Our findings reveal additional complexity of interaction between VEGF pathway molecules in ovarian cancer, and demonstrate the potential limitations of applying specific molecular techniques in a therapeutic setting.

#3) Examining the role of ErbB2 in a mouse model of IGF-IR induced mammary tumourigenesis

Campbell, C.I., Jones, R.A. and Moorehead, R.A. Department of Biomedical Sciences, OVC, University of Guelph

The type I insulin-like growth factor receptor (IGF-IR) and epidermal growth factor receptor 2 (ErbB2) are receptor tyrosine kinases extensively implicated in human breast cancer. The IGF-IR is involved in cell proliferation and survival and is overexpressed in a large proportion of breast cancers. We have previously created an inducible transgenic mouse model of IGF-IR overexpression in the mammary gland, in which tumours form. In addition, the RM11A cell line, isolated from tumour tissue was shown to maintain inducible IGF-IR overexpression. The purpose of this study was to examine the interaction between IGF-IR and ErbB2 using our model. In RM11A cells, survival was impaired upon downregulation and inhibition of ErbB2 *in vitro*. *In vivo*, overexpression of ErbB2 conferred a more aggressive phenotype to RM11A cells. In our transgenic mouse model, ErbB2 was upregulated in primary tumours overexpressing the IGF-IR and was downregulated upon removal of IGF-IR induction and also in IGF-IR-independent recurrent tumours. In conclusion, ErbB2 is important in IGF-IR-induced mammary tumourigenesis and can enhance tumour growth. Also, the IGF-IR appears to have a role in the regulation of ErbB2 in our model.

#4) The role of Akt isoforms in IGF-IR signalling and cancer progression in breast cancer

Sara Gagnon, Roger Moorehead, Department of Biomedical Sciences, OVC, University of Guelph

IGF-IR signalling has been linked to increased proliferation, and invasiveness in breast tumors. In our mouse model, which can selectively overexpress IGF-IR in the presence of doxycycline, we have found that overexpression of IGF-IR leads to mammary tumor formation while removal of the IGF-IR overexpression results in tumor regression in a majority of the tumors, a small percentage of which reoccur. Phosphorylated Akt has been found to be upregulated in these tumors and is known to be a primary mediator of IGF-IR signalling. There are three different isoforms of Akt and there is evidence that Akt1 promotes primary tumor growth but inhibits metastasis, while Akt2 promotes metastasis. To determine whether Akt isoforms differentially affect mammary tumor growth and migration, we have downregulated the Akt isoforms in a murine mammary tumor cell line, RM11A. Proliferation and migration of these cells were then evaluated *in vitro*. Primary and recurrent tumor tissues were examined to determine which Akt isoform is predominantly phosphorylated during differing stages of cancer progression. A better understanding of the role of Akt isoforms in IGF-IR signalling and in cancer progression could lead to more targeted therapies.

#5) Role of Nck adaptor proteins in endothelial cells during angiogenesis

Richard Harris, Nina Jones, Molecular and Cellular Biology, University of Guelph

Angiogenesis, the sprouting of new capillaries from pre-existing vessels, is crucial for the vascularization of newly developing organs, as well as the growth and metastasis of tumors in adults. This process is dependent on the migration of endothelial cells and their precursors, and is strictly regulated by intracellular signaling mechanisms that coordinate cellular behaviour through the actin cytoskeleton. Nck is a family of widely expressed adaptor proteins (Nck1 and Nck2) that bind activated receptor tyrosine kinases on the surface of endothelial cells, such as VEGFR-2, and signal to downstream effector proteins that organize the actin cytoskeleton network. Of note, developing tumors can secrete VEGF to stimulate angiogenesis in the surrounding vasculature through endothelial cell proliferation and migration, which contributes to their growth and maturation. In order to investigate the physiological role of Nck in endothelial cells, we have generated mice lacking Nck2 expression in embryonic endothelial cells of Nck1-null mice. Conditional deletion of Nck in endothelial cells leads to improper development of the cardiovascular system with prominent defects in the heart and yolk sac vasculature, resulting in embryonic lethality. Experiments are underway to determine the effect of Nck deletion on the actin cytoskeleton of cultured endothelial cells isolated from these mice. Our results to date imply a critical role for Nck proteins in the formation of the embryonic cardiovascular system, and suggest that Nck may be a promising molecular target for cancer therapy for inhibition of endothelial cell migration during angiogenesis.

#6) Effects of glucose concentration on the metabolic viability of mouse epithelial ovarian cancer (EOC) cells

Lisa Kellenberger[1], Alison Holloway[2] and Jim Petrik[1];[1]Biomedical Sciences, University of Guelph; [2] Department of Obstetrics and Gynaecology, McMaster University, Hamilton, ON.

Hyperglycemia is thought to be associated with an increased risk of cancer development. Cancer cells have high glucose requirements, in part because of a preference for glycolytic metabolism. We propose that high glucose may facilitate EOC tumour development by providing an abundance of fuel, and that anti-hyperglycemic drugs may be beneficial in EOC therapy. In preliminary investigations, a mouse-derived EOC cell line (ID8) was treated with 0 mM, 2 mM, 6 mM, 16.7 mM or 25 mM glucose. There was a dose-dependent increase in metabolic viability with increasing glucose, as measured by the MTT assay. The anti-hyperglycemic drugs rosiglitazone and metformin both led to a decrease in cell viability, independent of glucose concentration. Thus, these drugs appear to have direct cellular effects. Mechanistically, the effects of high glucose may be mediated by changes in expression of two families of glucose transporters, GLUT and SGLT. EOC cells over-express the GLUT-1 transporter and preliminary Western blot analysis has shown increased SGLT-2 expression in cancerous compared to normal human ovarian epithelium. We have detected GLUT -1 and -3 and SGLT -2 and -3 in ID8 cells by RT-PCR. Future work will investigate functional glucose uptake and transporter expression at varying glucose concentrations.

#7) The potential mechanism underlying VEGFR-2 heterogeneity in the tumor vasculature

Elizabeth Kuczynski, Brenda Coomber, Biomedical Sciences, OVC, University of Guelph

In clinical studies, anti-angiogenic cancer therapies, including those that target the VEGF signaling axis, produce minor, absent, or refractory effects on tumor growth and patient survival. The reasons for this variability are unclear. Previous work in our laboratory indicated that the pro-angiogenic VEGF receptor 2 is in fact heterogeneously expressed in the tumor endothelium. This finding potentially challenges the dogma of ubiquitous VEGFR-2 expression, its role in tumor biology, and complicates the notion of tumor vasculature inhibition. This project aims to determine the mechanism governing the heterogeneous expression of VEGFR-2 in tumor endothelium. Due to its pleiotropic roles in tumor progression and angiogenesis, TGF-beta1 was investigated for its role as a repressor of VEGFR-2. Preliminary results show that in endothelial cell lines bEND3 and BAEC, TGF-beta1 dose- and time-dependently reduced protein levels of VEGFR-2. Inhibition of TGF-beta receptor 1 did not inhibit this down-regulation. ChIP analysis will investigate transcription factor GATA-2-mediated regulation at the VEGFR-2 promoter, and further protein analysis will examine GATA-2 interaction with the repressor Hex. Additional studies will investigate: A) the mediators of TGF beta1-mediated VEGFR-2 down-regulation; B) the potential causes of differential TGF-beta1 activity within the tumor microenvironment; C) functional consequences of VEGFR-2-negative endothelium; and D) a tumor-bearing mouse model of VEGFR-2-repressed tumor endothelium. Ultimately, these results may provide insight on the efficient use of VEGF/VEGFR-2-targeted cancer therapies.

#8) Efficient knockdown of MMR proteins in human CRC cells using chained microRNA constructs

Kristen Lacombe, Brenda Coomber, Biomedical Sciences, OVC, University of Guelph

In colorectal cancer, microsatellite instability (MSI) is a frequent occurrence which causes an increase in mutation rate leading to tumor progression. Previous studies in our laboratory established that MSH2 expression is mediated by ischemia, and that this leads to *de novo* point mutations in the transforming oncogene KRAS. Since the cells previously used had been genetically manipulated to loose an activating allele of KRAS, we wished to create an *in vitro* model that is able to sufficiently mimic the mutator phenotype seen in MSI colorectal cancer earlier in progression (i.e. prior to KRAS activation). We achieved this by knocking-down the expression of a key mismatch repair proteins, MSH2. This was done by stably transfecting Caco-2 colorectal cells (which are mismatch repair proficient and microsatellite stable) with a plasmid (BLOCK-iT; Invitrogen) containing chained microRNAs (miRNAs) designed to target MSH2 mRNA. Cells were then seeded at low density in selection media containing blasticidin and different clones were isolated. Protein knock-down in each clone was then examined through western blotting. Greater than 80% protein knockdown was achieved through miRNA targeting of MSH2, and we found chained constructs were more effective than single miRNA sequences. Co-repression of MSH6 but not MLH1 was also observed in MSH2 knockdown cells. Further studies will be done to determine the MSI status of these cells as well as other molecular changes they have undergone. This study will further elucidate the effects of decreased MMR protein expression on cell behavior and aid in our understanding of its involvement in tumor progression.

#9) An interaction between SNAP23 and non-muscle myosin-IIA during cell adhesion

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Cell adhesion and migration is a fundamental process in multicellular organisms. It requires highly regulated cell-ECM interactions, a fact which is evident in the many diseases that can occur when these interactions are perturbed. A prominent example is seen when abnormal control of cellular proliferation and migration leads to metastatic cancers. Although much research has been done on cell surface and ECM components involved in cell motility, recent interest has focused on intracellular machinery that controls the location and movement of adhesive components within cells. In most membrane trafficking events, SNARE (Soluble NSF Attachment Protein Receptor) complexes are the major mediators of vesicle fusion. Through a series of studies conducted by our laboratory and others, a picture of how SNAREs function during cell motility is emerging. The regulation of SNARE complex assembly involves an array of factors; however, those that regulate SNAREs specifically during cell adhesion and migration have yet to be identified. Using LC-MS/MS analysis, an interaction between a plasma membrane SNARE (SNAP23) and Non-Muscle Myosin Heavy Chain-IIA (NMMHC-IIA) was detected. Investigation of the putative interaction between SNAP23 and NMMHC-IIA was performed using western blot analysis and confocal immunofluorescence microscopy. Furthermore, GFP-SNAP23 appears to co-localize with NMMHC-IIA on stress-fibres during cell spreading.

#10) Structure and sequence variation of the canine perforin gene

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Lymphocyte-mediated cytotoxicity is essential to control viral infections, limit lymphocyte expansion and activation, and survey for malignant cells. Humans with defects in lymphocyte cytoxicity have reduced perforin function resulting in uncontrolled histiocyte activation. Dog breeds such as Bernese mountain dogs (BMD) have a high incidence of reactive and malignant diseases affecting histiocytes. This study addressed the hypothesis that changes in the perforin gene contribute to histiocytic neoplasia (HN). Canine perforin DNA was amplified and sequenced through multiple PCR assays from healthy and diseased dogs, and the gene structure determined by rapid amplification of cDNA ends. The gene consists of 1679 bp, with two exons of 536 bp and 1143 bp, and an intron of 865 bp. Gene configuration and location differ profoundly from those in other species although the coding sequence remains conserved. The flanking sequence of the gene has similarity to a flanking area of the human perforin gene, suggesting an ancestral chromosomal translocation that may account for loss of the first non-coding exon. Three silent single nucleotide polymorphism (SNP) were identified. Analysis of the SNP distribution indicated a consistent genotype among 6 middle-aged to older BMD without HN. Among samples from 10 dogs with HN and 10 without HN, SNP occurred with variable frequency.

Conclusion: Changes in amino acid sequence of perforin were not associated with HN but silent gene mutations occurred at higher frequency in BMD with than without HN. Studies with a larger sample size are required to determine whether a specific genotype may characterize BMD without HN. Future studies will investigate the potential contribution of reduced perforin expression and function to HN in dogs.

#11) The use of lithium carbonate to prevent lomustine-induced thrombocytopenia in dogs: a pilot study

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Introduction: Lomustine is an anticancer agent of the nitrosourea class which is used in veterinary medicine to treat central nervous system tumors, lymphoma and mast cell tumours. A dose-limiting side-effect is cumulative thrombocytopenia. Lithium, a psychomodulating drug, stimulates hematopoiesis. We investigated if lithium would safely prevent lomustine-induced thrombocytopenia.

Methods: Four ~10 kg beagles received lomustine 20 mg PO q3weeks with cephalexin prophylaxis. Two dogs also received lithium 150 – 300 mg PO b.i.d. Hemograms, serum biochemistries, urinalyses and serum lithium levels were obtained weekly.

Results: Dilute urine (sp. gr. 1.000 – 1.020) developed within 1 month of starting lithium. Lithium levels were 0.57 ± 0.24 mmol/L in one dog (therapeutic range 0.5 – 1.5 mmol/L). In the second dog lithium level was 0.37 ± 0.09 mmol/L, but rose in week 13 to 1.89 mmol/L, causing depression and laboratory abnormalities: Hct - 0.74 L/L, Na - 138 mmol/L, ALT - 3244 U/L, and ALP - 1260 U/L. The dog was withdrawn and was normal within 1 month. The second dog receiving lithium had recurrent seizures beginning week 35. The dog was withdrawn week 39 and seizures stopped. All dogs developed Grade 1 - 4 neutropenia without sepsis after each lomustine treatment. In dogs receiving lomustine only, platelets dropped from 293 and 274 x 109/L in week 1, to 161 and 199 x 109/L in weeks 31 and 40, respectively. In dogs receiving lomustine and lithium, platelets dropped from 288 and 351 x 109/L in week 1, to 216 and 261 x 109/L in weeks 14 and 40, respectively.

Conclusions: Lithium does not appear to prevent lomustine-induced neutropenia and has important side-effects. Because of withdrawals, it was not determined if lithium prevents thrombocytopenia.

#12) Estrogen Metabolism in Normal Bone and Canine Osteosarcoma

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Osteosarcoma (OS) is the most common primary bone tumour in dogs and humans. At present prognosis remains poor. The relation of estrogens to the development and maintenance of bone is well established. Less is known, however, about estrogens with respect to bone cancer compared with their implication in other forms of cancer (e.g. breast, uterus). Since the effects of estrogens result in part from products that are formed and act locally, we examined estradiol (E2) metabolism in samples of normal and cancerous bone tissues. Radiolabelled estrogen was used in incubations to show clearly the extent of metabolism. Profiles for the steroids extracted from media were obtained by chromatography (HPLC) and revealed several features of interest: (1) water-soluble conjugates (30-40%), (2) major amounts of lipoidal estrogens - a potential storage form, and (3) possible presence of 2- methoxy-estradiol (2-MOE2). This latter finding is particularly significant because 2-MOE2 is a naturally occurring metabolite of E2 that has anti-angiogenic and anti-proliferative properties. As an anti-cancer agent its use is limited by difficulties in delivery to the intended site. Investigators in the UK have now succeeded in overcoming this problem by making a derivative of 2-MOE2 that has high bioavailability and is orally active. It is important nonetheless for us to establish whether 2-MOE2 is made in bone tissue and whether its production differs in osteosarcoma.

#13) Cannabidiol (a non-psychoactive component of cannabis) may act as a 5-HT_{1A} receptor agonist to reduce toxin-induced nausea and vomiting

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Cannabidiol (CBD), a non-psychoactive component of cannabis has been shown to suppress acute cisplatin-induced vomiting in shrews and reduce lithium-induced conditioned gaping to a flavour, but little is known about its mechanism of action, as CBD has very low affinity for the CB_1 and CB_2 receptors. Evidence suggests the 5-HT_{1A} receptor in the neuroprotectant and anxiolytic properties of CBD, but no such study has investigated the mediation of CBD's anti-emetic and anti-nausea properties. Experiments 1-3 investigated the effect of WAY100135 (a 5-HT_{1A} antagonist) and CBD on nicotine- (Experiment1), lithium- (Experiment 2), and cisplatin- (Experiment 3) induced emesis in shrews. Experiment 4 explored the ability of WAY100135 and CBD to interfere with the establishment of lithium-induced conditioned gaping in rats (a rodent model of nausea). At 5 mg/kg, CBD reduced nicotine-, lithium-, and cisplatin-induced vomiting in shrews, and interfered with the establishment of conditioned gaping in rats. At 10 mg/kg, CBD also reduced cisplatin-induced vomiting in the shrews. WAY100135 blocked the CBD-induced suppressant effects on vomiting and conditioned gaping. The anti-emetic and anti-nausea effects of CBD appear to be mediated by agonism of 5-HT_{1A} receptors. Most likely, CBD acts as an agonist on somatodendritic 5-HT_{1A} receptors on the raphe nuclei, serving to reduce the firing rate of 5-HT_{1A} afferents.

#14) Resistin, but not adiponectin is elevated in Caucasian prostate cancer patients

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Emerging evidence suggests that adipokines, such as resistin and adiponectin, play a role in modulating cancer risk. However, the role of resistin and adiponectin in prostate cancer (PCa) risk is poorly understood. 275 archived plasma samples consisting of 138 PCa cases and 137 controls were analyzed for resistin and total adiponectin protein using a Bio-Plex human adipokine assay and xMAP technology. Overall, resistin, but not adiponectin, was significantly elevated ($p=0.02$) in PCa patients compared with controls. Future studies are warranted to determine the functional role of resistin in PCa. When examined by ethnicity, there was no difference between cases ($n=16$) and controls ($n=8$) in African American subjects for either adipokine. In contrast, among Caucasians, adiponectin ($p=0.07$) and resistin ($p=0.03$) were higher in PCa patients ($n=114$) compared with controls ($n=110$). Our data suggests that differences in circulating adipokines may exist between ethnic groups, but this requires further study in a larger population. (D. Ma. is funded by an NSERC Discovery grant. B.K. Smith is funded by an OGS and Sun Life Financial Research Fund).

#15) The Thrombospondin-1 mimetic peptide ABT-510 increases the uptake of chemotherapeutics and induces regression of established epithelial ovarian tumours.

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Epithelial ovarian cancer (EOC) is the most common gynaecologic malignancy and current therapies have limited success. Thrombospondin-1 (TSP-1) is an endogenous anti-angiogenic protein and mimetic peptides such as ABT-510 have been created and are currently in clinical trials. We hypothesize that through vasculature normalization, ABT-510 will enhance tumour uptake of chemotherapeutics in an orthotopic syngeneic mouse model of EOC. Our model involves injecting tumourigenic epithelial cells under the ovarian bursa where they then colonize, grow tumours and eventually form ascites approximately 90 days post-injection. 60 days post-tumour induction animals were treated with ABT-510 to determine whether the peptide could induce regression of established tumours. Prior to sacrifice and tissue collection, some mice were injected intraperitoneally with tritiated paclitaxel to quantify the tumour tissue uptake of the chemotherapy drug following treatment with ABT-510. ABT-510 and chemotherapeutics caused a significant regression of established ovarian tumours and inhibited formation of secondary lesions. Animals treated with ABT-510 also had a significantly higher uptake of radiolabeled paclitaxel, suggesting that the peptide facilitated intratumoural delivery of the drug. Results from these studies demonstrate that ABT-510 has the ability to decrease primary tumour growth, inhibit abnormal tumour vascularity and increase the uptake of other cytotoxic agents.

#16) The role of the type I insulin-like growth factor receptor on NNK-induced lung tumorigenesis.

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The type I insulin-like growth factor receptor (IGF-IR) has been shown to be implicated in several types of human cancers, including lung cancer. In order to further investigate the role of IGF-IR in lung tumorigenesis, our lab has generated a doxycycline-inducible transgenic mouse model which over expresses IGF-IR in the lung tissue. This mouse model has allowed for the examination of a possible association between IGF-IR induced tumor formation and nicotine induced tumor development. Immunohistochemistry and western blotting were used to characterize and compare the tumors that develop in IGF-IR transgenic mice that were injected with NNK in comparison to those that were not injected. Analysis of tumor burden using H&E has shown that there is no difference in the number of tumors that develop. Western blotting has revealed an increased expression of IGFIR, P-Akt and P-CREB and P-p38 in the tumors of the NNK injected mice in comparison to the control mice, while no differences were found in the expression levels of Akt-1, Akt-2, and P-Erk. Similar results were found through the use of immunohistochemistry. Overall, these results suggest that IGF-IR overexpression does not induce more aggressive tumor formation in NNK derived tumorigenesis.

#17) Osteopontin and it Role in Tumorigenesis in the MTB-IGFIR Mouse Model

Devan Thompson, Roger Moorehead, Biomedical Sciences, OVC, University of Guelph

Osteopontin (OPN) is a secreted glycoprotein that interacts with a variety of cell surface receptors to increase migration, proliferation and survivability. Overexpression of OPN has been found in a variety of cancers, specifically breast cancers, with increased circulating levels of plasma OPN associated with increased tumour burden and worse prognosis. In our MTB-IGFIR mouse model, which utilizes a tet-on system to selectively overexpresses IGF-IR in mammary tissue, OPN mRNA has been found to up-regulated by 77-fold in mammary tumours compared to normal mammary tissue. To determine the in vitro effects of OPN on mammary tumour growth in our model RNAi will be used to down-regulate OPN in the RM11A murine tumour cell line. Cellular migration will be assessed using a migration assay; proliferation will be examined using ki-67 immunohistochemistry; and cell survival will be assessed using annexin-v immunohistochemistry. Additionally, a stable OPN knockdown cell line will be injected into the mammary glands of mice and used to study the effects of OPN on migration, proliferation and survival in vivo. This research hopes to expose the mechanisms through which OPN impacts tumour growth and help determine if OPN is a suitable candidate as a molecular target for cancer therapy.

#18) Canine subcutaneous mast cell tumours: Pathological characterization and prognostic indicators.

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Histological grading schemes for canine cutaneous mast cell tumours were not developed to evaluate subcutaneous mast cell tumours, which are currently categorized as grade II or higher. The aim of this investigation was to assess the pathology and clinical outcome for subcutaneous mast cell tumours to provide a more accurate prognosis. Cases were selected from YagerBest for the period 2002-2008. Information on clinical outcome was obtained from veterinarians and correlated with histological features. 309 cases fulfilling the inclusion criteria were evaluated. The dogs had a median age of 8 years (1.5 to 18 years) and mean and median follow-up was 842 and 891 days (2 to 2305 days). 156 (50.5%) dogs died during the study period, however only 30 (9.7%) were due to MCT. 98 (31.7%) were still alive at the end of the study. Metastasis occurred in less than 4% of dogs and 24(9.7%) had local reoccurrences, even though 56% of cases had incomplete surgical margins. Median survival time was not reached and estimated 6 month, 1,2 and 5 year survival probabilities were 0.91, 0.86, 0.81 and 0.58. Decreased survival times were associated with a mitotic index >5/10 HPF, incomplete surgical excision, reoccurrences, metastasis, nuclear diameter>5um and presence of necrosis. Both uni- and multivariate (Cox regression method) analysis showed mitotic index to be strongly predictive of survival. The results of the study indicate that the majority of subcutaneous mast cell tumours have a favorable prognosis, with extended survival times and low rates of recurrence and metastasis.

#19) Partial Purification of Porcine Cysteine S-Conjugate N-Acetyltransferase

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Mercapturic acids (N-acetylcysteine S-conjugates) were first reported as elimination products in 1879, but it was not until the late 1950's that the relationship between mercapturic acid formation and glutathione conjugation was established. In mammals, the mercapturic acid pathway represents a major route for the elimination and excretion of xenobiotics (*e.g.*, toxicants, carcinogens) and certain endogenous metabolites. Here we describe the partial purification of the enzyme responsible for generating these conjugates during detoxification. The goal is to purify the enzyme sufficiently to obtain a sample that can be characterized by Edman degradation or mass spectrometric sequencing. Based on the sequence data so obtained, we hope to identify the gene for NacT and evaluate is expression during carcinogen exposure.

#20) Pathogenesis and Oncogenesis of Sheep Betaretroviruses

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Enzootic nasal adenocarcinoma (ENA) and ovine pulmonary adenocarcinoma (OPA) are contagious tumors of sheep and goats affecting the nasal mucosal glands and secretory epithelial cells of the distal lung, respectively. Clinical symptoms include continuous mucosal discharge and respiratory distress. A retroviral etiology has been demonstrated for OPA with Jaagsiekte Sheep Retrovirus (JSRV) as the causative agent and a similar retroviral etiology for ENA has long been assumed. Studies have shown that tumors and fluids obtained from naturally affected sheep contain reverse transcriptase activity associated with a particle of buoyant density characteristic of retroviruses, identified as the enzootic nasal tumor virus (ENTV). However, definitive proof that ENTV is the etiologic agent of ENA has yet to be demonstrated. The envelope proteins of JSRV and ENTV have been identified as transforming agents *in vitro* but the mechanism of envelope mediated transformation is unclear. The full-length sequence for ENTV has been published, but no attempts to generate an infectious molecular clone have thus far been undertaken. Construction of a molecular clone of ENTV and its envelope protein represents a vital step toward the study of this animal model of epithelial-derived tumors and will enable further studies of the pathogenic and oncogenic mechanisms of ENTV.

#21) Mapping the Substrate-Binding Pocket of the P-Glycoprotein Multidrug Efflux Pump

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The ATP-binding cassette (ABC) protein, P-glycoprotein (Pgp, ABCB1), is a 170 kDa drug-efflux pump that has been implicated in the development in human tumours of multidrug resistance (MDR) towards cancer chemotherapeutics. Pgp is a polyspecific ATP-driven transporter which interacts with hundreds of compounds, including many anti-cancer drugs. The drug-binding pocket of Pgp appears to be large and flexible with at least two overlapping sub-sites, and the X-ray crystal structure and fluorescence studies indicate that it can bind two drugs simultaneously. Our goal is to map the binding pocket by covalently linking fluorescent drugs to the two functional transport sites of Pgp, the R-site and the H-site. The R-site drug, azido-tetramethylrosamine (Az-TMR) has been crosslinked to Pgp in a 1:1 drug:protein molar ratio, and the resulting Pgp-drug adduct has been characterized using fluorescence and biochemical approaches. The affinity of Pgp-Az-TMR for binding a second drug molecule has been determined for several different compounds, indicating which drugs can simultaneously bind to Pgp, and their cross-interactions. The fluorescence of the Az-TMR Pgp adduct has also provided insight into the local environment surrounding the R-site. Understanding the biochemical basis of drug binding will enable the design of selective Pgp inhibitors to reverse MDR.

#22) SNARE involvement in membrane type 1 matrix metalloproteinase (MT1-MMP) trafficking and cell invasion.

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Cell migration and invasion through an extracellular matrix (ECM) are critical components of the movement of tumour cells during the progression of cancer. Matrix metalloproteinases (MMPs) are a family of zinc dependent proteolytic enzymes that function to degrade the ECM and high expression levels of these proteases are considered characteristic of invasive tumour cells. MMPs are trafficked in vesicles to lamellipodia and invadopodia where they are either secreted (soluble MMPs) or remain membrane bound (MT-MMPs), catalytically cleaving ECM substrates and facilitating cellular invasion. The intracellular trafficking and secretion of MMPs is critical for ECM remodelling cell invasion. A protein family that has a major role in vesicle trafficking, SNAREs (soluble N-ethylmaleimide-sensitive factor activating protein receptors) function to localize vesicles to target membranes and recent evidence supports a role for SNAREs in MMP localization. Using an invasive fibrosarcoma cell line (HT-1080), we have shown a role for specific SNARES in trafficking membrane type 1 (MT1) MMP to the cell surface. The results demonstrate that SNARE-dependent traffic of MT1-MMP is an important factor in the regulation of MMP activity and facilitation of ECM degradation and cellular invasion.

#23) **Investigating the role of the ShcD Adaptor Protein in Brain Tumours**

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Characteristics of cancer include unregulated cellular proliferation, migration, and insensitivity to surrounding conditions, all of which can arise from abnormal cell signaling. Determining the biochemical mechanisms by which cell communication occurs is therefore pivotal to understanding diseases like cancer. Our investigations focus on one molecular factor in the signaling process, the ShcD intracellular adaptor protein. Proteins of the Shc family have been associated with various malignancies including glioblastomas and neuroblastomas. Although relatively little is known about the recently-discovered ShcD variant, its presence in the nervous system, involvement in skin cancer metastasis, and putative upregulation in glioblastoma multiforme indicate a potential role for this protein in brain tumours. Using two experimental approaches, we are testing the hypothesis that abnormal ShcD expression is associated with the development of cranial and neuro-endocrine malignancies. The intentions are i) to determine whether levels of this protein are indicative of a particular diagnosis or prognosis, and ii) to understand the mechanisms by which ShcD signaling participates in cancer. Answering these questions will offer further insight into the molecular nature of brain cancer and may reveal future diagnostic / prognostic factors and therapeutic targets for treatment of the disease.

#24) Thalidomide treatment of canine hemangiosarcoma

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Noncutaneous hemangiosarcoma, a malignant neoplasm of vascular endothelium is highly metastatic in dogs. The prognosis for long-term survival with surgery and adjuvant chemotherapy is poor. Hence, new therapies are needed such as thalidomide which, in addition to teratogenicity, has antiangiogenic properties which can inhibit the proliferation of blood vessels associated with tumour development.

Purpose: this study retrospectively reports the results of thalidomide therapy in canine hemangiosarcoma.

Materials: Nineteen dogs with histologically diagnosed hemangiosarcoma were treated at 100-400 mg/day thalidomide with a median dose of 8.7 mg/kg/day (range 3.7-19.7).

Results: Hemangiosarcoma was in the spleen (15 with 6 ruptured), right atrium (4), liver (1), and kidney (1). The dogs were: stage I (9 dogs), II (7 dogs), and III (3 dogs). One dog (stage II) was lost to follow up at 1,044 days and one dog (stage I) was alive at 1,210 days. The overall median survival for 17 dogs was 160 days (range 34-1,087 days).

Conclusions: Only limited efficacy data are available; however, this pilot study revealed prolonged responses in some patients. Optimal dose and schedule of administration remains to be determined; however, the absence of myelosuppression and significant adverse effects suggests thalidomide could be used with combination chemotherapy.

#25) Suppression of the CTP: Phosphoethanolamine Cytidylyltransferase (Pcyt2) Gene in Breast Cancer Cells

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Change in phosphoethanolamine pool size in tumor tissues is an important indicator for tumor prognosis and drug-therapy efficacy. Phosphoethanolamine is the substrate of the regulatory enzyme CTP:phosphoethanolamine cytidylyltransferase (Pcyt2) in the *de novo* biosynthesis of phosphatidylethanolamine (PE). Metabolic labeling with 14C-ethanolamine revealed a limited Pcyt2 activity in MCF-7 breast cancer cells, which led to an accumulation of phosphoethanolamine and a decrease in PE synthesis in comparison with MCF-10A mammary epithelial cells. The limited Pcyt2 activity was due to the significant reduction in Pcyt2 promoter activity in breast cancer cells. The CAAT-box at position -82/-71 bp and a proximal GC-rich region (–128/-93bp) that specifically binds Sp transcription factors regulate the basal promoter function. The tumor suppressor EGR1 targets the second GC-rich region (-153/-43 bp) and accounts for the elevated Pcyt2 activity in MCF-10A cells. MCF-7 cells barely express early growth response factor 1 (EGR1) but possess significant nuclear factor κB (NFκB) activity. NFκB interacts with Pcyt2 promoter within the region -227/-160 bp and the mutation of one NFκB site reduced the promoter activity only in MCF-7 cells. Together, these data demonstrate that EGR1 is the main stimulator of Pcyt2 gene expression. Its limited activity in breast cancer cells leads to less Pcyt2, which can result in the accumulation of the Pcyt2 substrate phosphoethanolamine. In the absence of EGR1, cancer cells maintain the basal Pcyt2 expression by the interaction of the proximal CAAT and GC regions and by elevated NFκB activity.