Celebrating the 35th Anniversary of the Terry Fox Marathon of Hope in the city where Terry Fox began his epic run across Canada.

Célébration du 35e anniversaire du Marathon de l’espoir de Terry Fox dans la ville où Terry Fox a débuté sa course épique à travers le Canada.
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Numbered boards & layout for poster presentations – Salons B, C, D
1. ROLE OF INTERFERON INDUCED GENES IN DIFFERENTIAL THERAPEUTIC RESPONSE IN HIGH GRADE SEROUS OVARIAN CANCER

Au, Katrina K.1, Liliane Meunier2, Juliana Josakhian3, Peter Truesdell4, Andrew Craig1,4, Anne-Marie Mes Masson2,5, Jeremy Squire3, Madhuri Koti1

Departments of 1Biomedical & Molecular Sciences, 4Cancer Biology & Genetics, Queen’s University, Kingston, ON, Canada; 2Centre de recherche du Centre hospitalier, 5Department of Medicine, University of Montreal, Montreal, QC, Canada; 3Department of Genetics and Pathology Faculdade de Medicina, Ribeirão Preto, Sã Paulo, Brazil

Introduction: Resistance to platinum-based chemotherapy remains a major impediment in the treatment of high-grade serous epithelial ovarian cancer (HGSC). The dual roles of cancer immunoediting via tumour-promoting inflammation and suppression are well recognized. We hypothesized that a distinct tumour inflammatory microenvironment could determine therapeutic response in HGSC. The current study was performed to identify the role of interferon-induced genes as markers of primary (innate) chemotherapy resistance in HGSC.

Methods: Targeted inflammation gene profiling of chemo naïve fresh frozen tumour tissues revealed a signature of 11 genes predictive of therapeutic response. First phase validation of STAT1, the most significantly differentially expressed gene on an independent cohort of 183 HGSC cases using immunohistochemistry confirmed its role as a response predictive biomarker. Interestingly, STAT1 expression levels were positively correlated with the density of tumour infiltrating CD8+ lymphocytes. Phase 2 validation of STAT1 as a response predictive biomarker, evaluation of intra-tumoural CD8+ T lymphocytes, as well as validation of an interferon gene signature was performed on a larger cohort of HGSC patients accrued from the TFRI-OCC-Canadian Ovarian Experimental Unified Resource.

Results: Preliminary analysis indicated a correlation between STAT1 and CD8+ T cell infiltration.

Conclusions: Preliminary analysis revealed a strong correlation between STAT1 expression and intra-tumoural CD8+ T cell infiltration. Interferon induced STAT1 mediated alterations in the primary HGSC tumour immune microenvironment contributes to a variation in therapy response. These findings provide evidence for novel immune based response predictive biomarkers that need further validation in larger cohorts.

Outcome/Impact: Findings from the current study will aid in patient stratification based on expression of these markers. Furthermore, this study provides a basis to the understanding of interferon induced genes and their role in modulation of the tumour microenvironment.
2. THE DEVELOPMENT OF CTDNA BIOMARKERS FOR FORME FRUSTE TUMOURS

Bushell, Kevin\textsuperscript{1}, Miguel Alcaide\textsuperscript{1}, Sepideh Alamouti\textsuperscript{1}, Bruno Grande\textsuperscript{1}, David Huntsman\textsuperscript{2} and Ryan D. Morin\textsuperscript{1}

\textsuperscript{1}Department of Molecular Biology & Biochemistry, Simon Fraser University; \textsuperscript{2}Centre for Applied Genomics and Therapeutics, BC Cancer Agency.

Introduction: The detection of circulating tumour DNA (ctDNA) holds great promise as a non-invasive biomarker for solid cancers. We are exploring this potential in a group of individually rare cancers known as forme fruste tumours, each of which is defined by significant inter-tumour genetic homogeneity.

Methods: Each forme fruste tumour typically contains a highly recurrent driver mutation. Over 97% of cases of adult granulosa cell tumours (GCT) of the ovary harbour the C134W substitution mutation in FOXL2. To detect ctDNA in these cases, we will use the OnTarget™ mutant allele enrichment platform (Boreal Genomics), a highly sensitive method for enriching and quantifying hotspot mutations in the plasma. Other tumour types, such as synovial sarcoma and Ewing’s sarcoma are characterized by specific semi-recurrent chromosomal rearrangements. To detect these rearrangements, we combine optimized library preparation methods using custom single-molecule tagging adapters and an in-solution hybridization-based capture using biotinylated RNA baits that target the intronic regions that commonly harbour rearrangement breakpoints for targeted deep sequencing. Using these methods, we are determining which forme fruste cancers are associated with quantifiable levels of ctDNA, thereby nominating them as diseases in which ctDNA may be a useful biomarker. In parallel, we are quantifying rearrangement breakpoints using digital PCR to determine the accuracy of our methods.

Results: Analyses indicate that our library preparation method captures up to 41% of the input DNA molecules from a plasma sample. The hybridization protocol provides up to 50,000-fold enrichment of the targeted intronic regions. We are currently using these methods to quantify chromosomal rearrangement breakpoints in tumour and plasma samples from patients with various soft-tissue sarcomas including Ewing, Synovial and Myxoid liposarcoma.

Conclusions: Preliminary results suggest that our methods are promising and enable the accurate detection of tumour-specific breakpoints in plasma DNA.

Outcome/Impact: The potential utility of ctDNA as a generic biomarker for solid cancers is immense and this project will leverage the genetic homogeneity of the forme fruste tumours to evaluate this potential across these cancer types.

This research is funded by the TFRI and the BC Cancer Foundation.
3. SRC FAMILY KINASE, FELINE GARDNER-RASHEED (Fgr), IS OVEREXPRESSED IN ZAP-70 POSITIVE CHRONIC LYMPHOCYTIC LEUKEMIA

Ciapala, Alexandra C.¹, Dielschneider-Delong, Rebecca², Johnston, James B.², Gibson, Spencer B. ¹,²,³

¹University of Manitoba; ²CancerCare Manitoba; ³Manitoba Institute of Cell Biology

Introduction: Chronic Lymphocytic Leukemia (CLL) is the most common adult leukaemia in North America. It is characterized by an accumulation of monoclonal, CD19+, CD5+, and CD23+ B-cells in the blood, lymph nodes, and bone marrow. Standard therapies are initially effective, but drug resistance often occurs, and CLL remains incurable. One of the most important prognostic marker for CLL patients is the expression of Zeta-chain Associated Protein 70 kinase (ZAP-70). CLL cells with over-expression of ZAP-70 (ZAP-70(+)) are associated with shorter overall survival and more aggressive disease. The Src family kinase, Feline Gardner-Rasheed (Fgr), is another tyrosine kinase expressed in CLL cells and has been shown to play a role in metabolism which is known to be abnormal in ZAP-70(+) CLL cells. The role of Fgr in ZAP-70(+) CLL cells is unknown.

Methods: We used western blotting to determine protein levels. Immunofluorescence and cellular fractionation were used to determine the sub-cellular localization. Cell death was measured with Annexin V/7AAD staining by flow cytometry.

Results: We lysed ZAP-70(+) and ZAP-70(-) CLL cells and western blotted for Fgr. We found that Fgr expression was significantly increased in ZAP-70(+) CLL cells compared to ZAP-70(-) CLL cells. As a control, we determined the expression of metabolic proteins such as pyruvate kinase muscle isoform 2 and catalase and found that their expression remained unchanged. This indicates that Fgr expression correlates with increased ZAP-70 in CLL cells. We then determined localization of Fgr in CLL cells by immunofluorescence. We found that Fgr co-localizes in part with the mitochondria with the rest localized to the cytoplasm in ZAP-70(+) CLl cells. In contrast, Fgr failed to localize with the mitochondria in ZAP-70(-) CLL cells. This suggests that Fgr might be regulating metabolism in ZAP-70(+) cells. We treated CLL cells with the metabolic inhibitor Shikonin and found that ZAP-70(+) CLL cells undergo apoptosis to a greater extent than ZAP-70 (-) CLL cells. This indicates alteration in metabolism in ZAP-70(+) CLL cells.

Conclusions: Our results suggest a role for Fgr in altering the cellular metabolism in ZAP-70(+) CLL cells.

Outcome/Impact: Metabolism might be a novel target for treatments for aggressive forms of CLL.
4. SURGERY-INDUCED VACCINE DYSFUNCTION IN A MURINE B16 MELANOMA MODEL

Lansdell, Casey 1,2, Lee-Hwa Tai 1,2, Abhirami Ananth 1,2, Almohanad Alkayyal 1,2, Christiano de Souza 1, John Bell 1,2, Rebecca Auer 1,3

1Center for Innovative Cancer Research, Ottawa Hospital Research Institute, Ottawa, ON; 2Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, ON; 3Department of Surgery, Division of General Surgery, The Ottawa Hospital, Ottawa, ON

Introduction: Cancer surgery results in an immunosuppressive environment that promotes tumour growth. Preclinical studies from our lab have demonstrated that complete protection from tumour challenge, conferred by a tumour-associated antigen (TAA)-encoded vaccine, is rendered ineffective post-surgery in a murine melanoma model. The objective is therefore to evaluate the impact of surgical stress on TAA-specific adaptive T cell immunity, specifically the number, distribution and function of antigen-specific cytotoxic T lymphocytes (CTLs) in a murine B16 melanoma model of surgical stress.

Methods: Prophylactic model: C57BL/6 mice were immunized with AdhDCT, adenovirus expressing human dopochrome tautomerase (hDCT), a melanoma TAA. 7 days later, mice underwent B16 tumour challenge and remained untreated or endured an abdominal nephrectomy to induce surgical stress. DCT-specific CTL number by was quantified by DCT-loaded MHC1 tetramer and function by intracellular staining of IFNγ, TNFα and Granzyme B by flow cytometry 1 day post-surgery. Proliferation was assessed by BrdU incorporation. Therapeutic Model: B16 cells were implanted 7 days pre-AdhDCT and 14 days pre-surgery. DCT-specific IFNγ and TNFα production and CD137 activation was determined by flow cytometry as well as Gr-1+CD11b+ myeloid derived suppressor cells (MDSCs) and CD4+CD25+FoxP3+ regulatory T cells (Tregs).

Results: Surgery does not alter the proportion of DCT- specific CTLs but does significantly attenuate their function via IFNγ, TNFα and Granzyme B production. A postoperative attenuation in proliferation, but not activation, was observed. Surgery, not the tumour, results in an accumulation of MDSCs and Tregs.

Conclusion: Our results suggest that surgical stress does not impair DCT-specific T-cell activation, but does attenuate proliferation and function associated with preoperative vaccination. This may be attributed to surgery-induced MDSCs and Tregs, well-known immune suppressors.

Impact: Surgical resection is the leading treatment of most solid tumours but surgical stress creates an immunosuppressive environment that promotes tumour recurrence and metastases. Understanding the mechanisms of T cell dysfunction following surgery will facilitate the development of targeted immunotherapies to reverse this effect in the postoperative period.
5. MECHANISMS AND IMPACTS OF EXOSOMAL MICRORNAS ON LUNG ADENOCARCINOMA TUMOURIGENESIS

Lawson, J.¹, Dickman C.¹, Lam S.¹, Garnis C.¹

¹Department of Integrative Oncology, BC Cancer Research Centre, Vancouver

Introduction: Lung adenocarcinoma (LA) is a leading cause of cancer death worldwide and a better understanding of LA biology could improve outcomes for patients with this disease. Exosomes are membrane bound vesicles that are released from many different cell types including cancer cells. Recently microRNAs (miRNA), which are non-coding RNAs that function in post-transcriptional gene regulation, were discovered to be packaged within exosomes in a selective fashion. However the mechanism and function of these exosomal miRNAs are not well understood. We aim to determine novel mechanisms driving exosomal miRNA packaging and the function of miRNAs enriched within LA exosomes.

Methods: miRNA profiles were generated from exosomal and cellular fractions of five LA cell lines, using qRT-PCR on a panel of 742 miRNAs. Candidate miRNAs with at least a four-fold change between the two fractions were selected for analysis. miRNAs enriched in the exosomal fractions were analyzed with MEME suite, a motif-based sequence analysis tool, to determine whether mature miRNA sequences shared a common motif. To assess the biological role of the miRNA candidates, lentiviral miRNA inhibitors and mimics were used.

Results: We detected the expression of 264 miRNAs in the exosomes and 258 miRNAs in the cells. An average of 15 were observed to be selectively released in exosomes and 14 were selectively retained in cells. Comparing across all cell lines, 8 miRNAs were found to be enriched in at least 4/5 exosome samples relative to the cells, while only one miRNA was selectively retained in at least 4/5 cell lines relative to the exosomes. These miRNAs were also found to be deregulated in LA tissues. Identified exosomal miRNAs appear to regulate key oncogenes, including EGFR and c-Myc. Exosomal miRNAs were enriched for a known RNA binding motif UGUA.

Conclusion: We have identified a set of miRNAs that are commonly enriched in LA cell line exosomes. These exosomal miRNAs contain a UGUA binding motif, which may play a role in their exosomal localization.

Impacts: New knowledge in exosome biology and miRNA-mediated cancer processes may be exploited for novel diagnostic, preventive, and therapeutic approaches for use in the clinical setting.

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6. CRK ADAPTOR PROTEIN SIGNALLING IN BREAST CANCER TUMOURIGENESIS

Shergill, Amrita, Emily S. Bell, Kelly E. Fathers, William J. Muller, Morag Park

Goodman Cancer Research Centre, McGill University, Montreal, Quebec

Introduction: Crk proteins function as adaptors that integrate upstream extracellular signals with downstream signalling networks. Crk proteins are overexpressed and amplified in multiple human cancers and increased Crk levels are associated with high-grade breast cancers and poor outcome in human breast cancer datasets. We aim to determine the mechanistic role of Crk proteins in cancer by identifying key signaling pathways involved in Crk-dependent tumourigenesis, in the basal and ErbB2 amplified (ErbB2+) subtypes of breast cancer.

Methods: We created a number of stable Crk knockdown (KD) and over-expression (OE) cell lines using shRNA and performed numerous in vitro and in vivo techniques including tumour-sphere and proliferation assays. To address the contribution of Crk to ErbB2+ tumourigenesis in vivo we generated a transgenic mouse model where mammary tumours dependent on ErbB2 overexpress CrkI.

Results: Knockdown (KD) of Crk protein levels in aggressive basal breast cancer cells abrogated tumour formation in vivo. From this, we hypothesize that Crk proteins are required for breast cancer tumour propagation. We have established that Crk KD in human basal breast cancer cells significantly reduces tumour-sphere forming capacity of tumour-initiating cells (TICs) in vitro.

Following Crk KD in three ErbB2+ breast cancer cell lines a significant decrease in TIC capability, a significant reduction in proliferation, and a less adhesive phenotype is observed in vitro. We have demonstrated that knockdown of all Crk isoforms has a more significant effect than knockdown of each isoform alone. In vivo results show that CrkI overexpression increases tumour penetrance in ErbB2+ dependent tumours. Tumour pathology of the CrkI-ErbB2+ mouse model is different to the adenocarcinoma-type of the ErbB2+ model, but similar to the basal-like CrkI overexpressing model.

Conclusions: Increased Crk expression in breast tumours culminates in a highly proliferative, aggressive basal tumour cell phenotype. Crk signalling in ErbB2-amplified tumour cells promotes a basal-like phenotype.

Impact: Since Crk proteins are elevated in many human cancers, these studies will not only provide an understanding of the role of Crk in poor outcome breast cancers also to our understanding of Crk in other cancers, leading to potential clinical therapeutic targets for treatment of patients with cancers expressing elevated Crk levels.
7. THE GENOMICS OF DRUG RESPONSE IN HEAD AND NECK CANCER

Sun, Ren X.1,2, Anthony C. Nichols3, Constance Li1,4, Nicholas J. Harding1, David Garcia1, Julie Livingstone1, John W. Barrett3 and Paul C. Boutros1,2,4

1Informatics & Bio-computing Program, Ontario Institute for Cancer Research, Toronto; 2Department of Pharmacology & Toxicology, University of Toronto; 3Department of Otolaryngology Head & Neck Surgery, Western University, London, Ontario; 4Department of Medical Biophysics, University of Toronto

Introduction: Treatment strategies for head and neck cancer (HNC) are poorly defined. The recently-established causal link to human papilloma virus (HPV) infection and HPV-driven differences in treatment response demonstrate a need for more targeted therapies.

Methods: Exome sequencing was carried out on 33 HNC cell lines, eight of which were derived from HPV-positive tumours, to identify somatic single nucleotide variants. Of these, 30 cell lines were assayed using Affymetrix OncoScan arrays to profile copy number aberrations (CNA). Drug screening, currently in-progress, is being conducted using four drug panels, totalling nearly 5000 compounds and exhibiting minimal overlap as well as including many drugs with previous clinical usage and history.

Results: We have identified clear mutational patterns in TP53, CDKN2A and PIK3CA between HPV-positive and HPV-negative cell lines that reflect those in primary tumours and are also highly concordant with our previous findings. Our preliminary CNA analyses suggest homozgyous deletion events of CDKN2A and amplification events of PIK3CA and SOX2 in HPV-negative cell lines. Our drug screening studies reveal several compounds with increased activity in HNC cell lines.

Conclusions: The differences observed in mutational and copy number events as a function of HPV-status suggest that HPV-positive and -negative tumours exhibit molecularly-distinct behaviours and should be treated as such clinically. Further refinement of our CNA analysis and integration of our drug screening results will provide additional insights into understanding drug sensitivity at the genomic level and target therapies for patients.

Outcome/Impact: Drug resistance and coarsely-defined therapeutic approaches contribute to a high recurrence rate of 50% within the first two years for HNC. Both the disease and its treatment affect intimate aspects of patients' lives. The results of this project will expand on current treatment options to improve overall prognosis of the disease.
8. USING ALDH1A3 INHIBITORS TO TARGET BREAST CANCER STEM CELLS

Thomas, Margaret¹, Krysta Coyle¹, Melissa Wallace¹, Michael Giacomantonio¹, Carman Giacomantonio¹,², Paola Marcato¹

Departments of Pathology¹ and Surgery², Dalhousie University, Halifax, NS, Canada.

Introduction: Breast tumours contain a population of cells known as cancer stem cells (CSCs) which are highly tumourigenic and resistant to typical chemo and radiotherapy. Aldehyde dehydrogenase (ALDH) isoform ALDH1A3 is selectively expressed in these breast cancer stem cells and ALDH activity is used to identify CSCs. ALDH1A3 plays a role in breast tumour progression as this enzyme initiates retinoic acid (RA) signaling and induces RA-inducible genes. A compound that inhibits these key signaling pathways could be used as a potential adjuvant therapy to target breast CSCs.

Methods: Twelve general ALDH inhibitors were tested for their effectiveness in targeting ALDH1A3 activity by quantifying their ability to decrease expression of RA-inducible genes in MDA-MB-231 and MDA-MB-468 breast cancer cells. Ability to directly inhibit ALDH activity was quantified with the Aldefluor assay on a patient tumour xenograft and on cell lines with known ALDH activity. To investigate the anti-cancer properties of these compounds, MDA-MB-231 and MDA-MB-468 cell lines were treated with the compounds and then apoptosis quantified with the annexin V assay. Finally, MDA-MB-231 cells implanted in female NOD/SCID mice are treated with citral to test if citral inhibits ALDH1A3-dependent tumour growth.

Results: Citral, disulfiram, and diethylaminobenzaldehyde significantly reduced ALDH1A3-mediated expression of at least one of the RA-inducible genes in cell lines. Citral and benomyl significantly reduced the ALDH activity of the xenograft, and reduced aldefluor activity of ALDH1A3 overexpression MDA-MB-231 cells. Citral and disulfiram induced apoptosis in an ALDH1A3-dependant manner.

Conclusions: Should citral prove to be an effective breast cancer and ALDH1A3 inhibitor in tumour xenograft experiments, this compound could potentially be used as adjuvant therapy for patients with tumours that have a large population of high-ALDH1A3 CSCs.

Outcome/Impact: These patients often have poor outcomes and recurrence, which has been attributed to the high number of CSCs present in their tumours. Therefore, targeting CSCs is a desirable goal in treating these patients.
9. PROMOTION OF PROTOPORPHYRIN IX (PpIX) FLUORESCENCE BY MEK INHIBITION IN IN VIVO CANCER MODELS

Yoshioka, Ema, Ann Dorward and Ken Hirasawa

Division of BioMedical Sciences, Memorial University, St John’s, Newfoundland

Introduction: Protoporphyrin IX (PpIX) is an endogenous fluorescence that is accumulated in cancer cells treated with the heme precursor 5-aminolevulinic acid (5-ALA). The cancer-specific fluorescence of PpIX is used to distinguish tumour from normal tissue, which has proven clinically useful during fluorescence-image guided surgery. Moreover, red light irradiation of cancer cells with PpIX accumulation generates reactive oxygen species and effectively induces cancer cell apoptosis, which is also known as the photodynamic therapy (PDT). In this study, we sought to investigate whether modulation of oncogenic Ras/MEK pathway mediates PpIX accumulation in cancer cells.

Methods: For in vitro studies, human breast, lung and prostate cancer cells were treated with or without the MEK inhibitor (U0126) for 20 hours, followed with 5-ALA for 5 hours. The cells were lysed, and the PpIX fluorescence accumulated in the cells was measured with a microplate fluorescence reader (λex 405 nm; λem635 nm). For in vivo studies, the 4T1 mouse breast cancer cells were injected into the right hind flank of 8 week-old Balb/c female mice. U0126 and 5-ALA were injected intraperitoneally for 5 and 2 hours, respectively. The tumours were removed and lysed for measurement of PpIX fluorescence.

Results: In vitro, MEK inhibition increases PpIX fluorescence in human cancer cell lines, but not in normal cell lines. The promotion of PpIX fluorescence by MEK inhibition was most commonly observed among human breast cancer cells. In vivo, PpIX accumulation was observed significantly more in the tumours from mice treated with U0126 and 5-ALA (2.4 fold) than those from mice with vehicle control (DMSO/saline) and 5-ALA.

Conclusions: MEK activation reduces PpIX accumulation in human cancer cells. In vitro and in vivo treatment of the MEK inhibitor significantly increases the accumulation of PpIX fluorescence in cancer cells.

Outcome/impact: Since the FDA has recently approved MEK inhibitors for cancer treatment, it would be a feasible idea to use the MEK inhibitor to enhance PpIX fluorescence for fluorescence-image guided surgery and to improve the efficacy of PDT treatment. As a next step, we will identify the molecular mechanism involved in the PpIX accumulation by MEK inhibition.
10. INVESTIGATION OF THE ANTIPLATELET AGENT TICAGRELOR AS A TREATMENT FOR BREAST CANCER METASTASIS

Brien, Colin D.¹, Gebremeskel, Simon¹, Levatte, Terry¹; Johnston, Brent¹, Bezhuly, Michael¹,²

¹Department of Microbiology & Immunology, Dalhousie University, Halifax, NS, Canada; ²IWK Health Centre, Halifax, NS, Canada

Introduction: Current evidence shows that platelets support tumour metastasis. Within the circulatory system, platelets guard tumour cells from immune elimination and promote their arrest at the endothelium, supporting the establishment of secondary lesions. Ticagrelor, currently in use for prevention of further thrombotic events following acute coronary syndrome and ischaemic stroke, is a novel P2Y12 antagonist that restricts the propagation of activated platelets.

Methods: Here we propose the use of ticagrelor as a means to inhibit breast cancer metastasis in the 4T1 murine breast tumour resection and experimental metastasis model by restricting the mechanisms described above. As well, platelet interactions with breast cancer cells and platelet-cancer cell emboli interactions with endothelium will be examined in the presence of ticagrelor in vitro.

Results: Compared to control, ticagrelor significantly (p<0.001) reduces metastatic 4T1 colony forming units in the lungs of mice. Also, ticagrelor results in a significant (p<0.01) increase in overall survival of mice in a 4T1 breast tumour resection model.

Conclusions: Ticagrelor is an antiplatelet agent that demonstrates a promising role as a treatment for metastatic breast cancer in mice, which has potential to translate clinically.

Outcome/Impact: Given that we have already observed that ticagrelor confers greater survival in mice, the ability of ticagrelor to prevent platelets from interacting with cancer cells should confer a greater survival advantage in human breast cancer patients. The knowledge gained from these studies will help in the design of clinical trials and could provide new therapeutic approaches to control a variety of malignancies that is not only limited to breast cancer.
11. P16INK4A-ASSOCIATED SENESCENCE IS A MAJOR THERAPY RESPONSE IN HUMAN OVARIAN CANCER CELLS

Calvo, Gonzalez LL1,2, Cheng S1,2, Skulimowski M1,2, Portelance L1, Clément I1, Provencher D1,2, Mes-Masson AM1,2, and Rodier F1,2.

1CRHUM, Institut du Cancer de Montréal; 2Université de Montréal

Introduction: Despite surgical and chemotherapeutic options, advanced ovarian cancer (OvCa) is the deadliest gynaecologic malignancy. We propose that understanding the cells fate decisions like death or senescence that are taken by cancer cells after chemotherapy could reveal new therapeutic opportunities. Based on previous preliminary data, our hypothesis is that high grade serous OvCa cells mostly undergo senescence, a strong tumour suppressor growth arrest. We propose to characterize this senescence cell fate decision and to identify the molecular players that regulate it.

Methods: We use OvCa primary cultures from the CRCHUM biobank to monitor different senescence hallmarks including: The senescence associated beta galactosidase (SA-β-Gal), the expression of cyclin-dependent kinase inhibitors (immunofluorescence), and permanent cell cycle arrest (EdU labelling index). The same hallmarks are also evaluated in response to therapy (carboplatin/paclitaxel/X-Ray) in cultured primary cells. Using tissumicroarrays (TMAs) built from pre- and post-treatment human OvCa tissue samples, we also test whether tumours can display senescence hallmarks in vivo.

Results: Using OvCa primary cultures from the CRCHUM biobank we find that 90% of them undergo culture-stress growth arrest. We find that primary OvCa cells readily undergo replicative senescence when maintained in culture: they stop proliferating, become enlarged and flattened, and acquire other senescence hallmarks including p16INK4a, a cyclin dependent kinase inhibitor that permanently prevent cell cycle progression. Chemotherapy-treated OvCa cultures similarly acquire the same senescence hallmarks. In patient-derived TMAs, we detect altered p16INK4a levels and reduced ki-67 (proliferation marker) following chemotherapy. High p16INK4a expression also correlated with a delayed progression of the disease.

Conclusion: Our data reveal that primary OvCa cells undergo p16INK4a-associated senescence in response to chemotherapy in vitro and in vivo and that this senescence response could be beneficial for the patient.

Outcome/Impact: Because the impact of resident senescent cells still remains controversial in the context of cancer treatment, it will now be important to determine how this phenomenon impact OvCa patient survival. If it turns out that senescence is beneficial, futures combined therapies could try to stimulate this cell fate in treated OvCa cells.
COMBINING TARGETED MICROTUBULE DESTABILIZING AGENTS AND ONCOLYTIC VIRUS VSVD51 IMPROVES VIRUS SPREAD IN RESISTANT CANCER CELLS

Garcia, Vanessa¹,², Nicole Forbes¹, Rozanne Arulanandam¹, Oliver Varette², Jean-Simon Diallo¹,²

¹Ottawa Hospital Research Institute, Ottawa, ON, Canada; ²Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

Introduction: Oncolytic viruses (OVs) specifically target and lyse cancer cells while leaving normal cells unharmed. Past work has shown that oncolytic VSVD51 replication in resistant cell lines can be achieved by combining VSVD51 with microtubule destabilizing agents (MDAs) which work by decreasing interferon (IFN) secretion and increasing mitotic catastrophe following virus infection. Despite the success of this strategy, the narrow therapeutic window of MDAs remains a hindering limitation. The use of more targeted MDAs, such as antibody-drug conjugates (ADCs), composed of a monoclonal antibody targeting a tumour antigen and a microtubule-targeted cytotoxic payload released upon antibody internalization, allow for specific drug delivery thus overcoming MDA-associated toxicities. The combination of VSVD51 with ADCs as a means to improve OV spread in resistant cancer cell lines will be examined.

Methods: The impact of combining VSVD51 with an MDA-antibody conjugate was assessed using a high-throughput virus titration method and cell viability was also examined using a metabolic assay. The mechanism of action of the ADC in the context of OV enhancement was probed by IFN-β ELISA and immunofluorescence, the former looking at the impact of the drug on the secretion of antiviral IFN-β and the latter assessing mitotic catastrophe.

Results: The VSVD51-ADC combination treatment was found to increase viral titers and enhance virus-associated cytotoxicity in an OV-resistant cancer cell line. Mechanistically, the ADC impairs IFN-β secretion upon VSVD51 infection thus dampening the cell’s antiviral response. Additionally, an increase in mitotic catastrophe quantified by cellular polynucleation is observed in the combination treatment, suggesting a bystander killing effect.

Conclusions: Combination treatment of VSVD51 with an ADC was shown to improve virus spread in a resistant cancer cell line but not a normal cell line.

Outcome/Impact: Improving the efficacy of OVs has been a challenge in the field. We have proposed a strategy that allows for the combination of VSVD51 with a clinical chemotherapeutic to improve OV replication while maintaining tumour specificity and minimizing treatment toxicities.
13. VITAMIN C INDUCED EPGENOMIC REMODELLING IN HOXA9-IMMORTALIZED BONE MARROW CELLS

Mingay, M.1, Chaturvedi A2, Moksa A1, Keith Humphries3 Heuser M2, Hirst M1,4

1Department of Microbiology and Immunology, UBC and the Centre for High-Throughput Biology; 2Department of Hematology, Hannover Medical School, Hannover, Germany; 3Terry Fox Laboratory, BC Cancer Agency Research Centre, Vancouver; 4Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency

Introduction: The epigenomes of many cancers are characterized by abnormalities in the distribution of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). Vitamin C (ascorbic acid; AA) has recently been shown to alter DNA methylation homeostasis by stimulating the catalytic activity of the ten-eleven-translocation (TET) enzymes. TET enzymes catalyze the oxidation of 5mC to 5hmC, an intermediate reaction in the de-methylation pathway of 5mC. Neomorphic mutations in IDH1/2 contribute to oncogenesis by producing the metabolite (D)-2-hydroxyglutarate (D-2HG), which inhibits TET. We aimed to explore the landscape of 5mC and 5hmC in a myeloid malignancy model expressing IDH1 R132H in the presence and absence of AA.

Methods: HOXA9-immortalized primary mouse bone marrow cells were transduced with an empty control, wild type IDH1 or IDH1 R132H vector and were grown in the presence or absence of AA (100ug/ml). Genomic DNA was extracted from IDH1 R132H mutant and control cells +/− AA after 72 hours and subjected to DNA immunoprecipitation sequencing using anti-5mC (meDIP-seq) or anti-5hmC (hmeDIP-seq) antibodies.

Results: 72 hours of AA treatment led to a 4-fold reduction in cell proliferation across all cell types regardless of IDH1 status. To identify epigenetic changes mediated by TET in response to AA treatment we defined a set of genomic regions that demonstrated a decrease of 5mC with flanking increases in 5hmC upon treatment (differentially methylated regions (DMRs). These AA responsive DMRs overlapped with transcription factor binding elements for the myeloid-specific transcription factors PU.1, RUNX1 and GATA1 at significantly greater (2.5-3 fold) frequency compared to randomized control regions of the same size. Gene ontology analysis revealed that regulatory regions gaining 5hmC and losing 5mC upon AA treatment are associated with genes that become down regulated in hematopoietic precursors constitutively expressing the HOXA9-NUP98 and HOXA9-MEIS1 fusion oncogenes.

Conclusion: These findings implicate AA as a natural compound capable of altering the epigenetic abnormalities associated with transformed myeloid cells to facilitate differentiation and apoptosis in our clinically relevant leukemia model.
14. GENETIC ALTERATIONS OF THE MHC CLASS II TRANSACTIVATOR CIITA ARE FREQUENT IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

Mottok, Anja1, Bruce Woolcock1, Fong Chun Chan1, Adéle Telenius1, Elizabeth Chavez1, Lauren Chong1, Merrill Boyle1, Susana Ben-Neriah1, David Scott1, Lisa Rimsza2, Reiner Siebert3, Randy Gascoyne1 and Christian Steidl1.

1Department of Lymphoid Cancer Research, BC Cancer Agency, Vancouver, BC, Canada; 2Institute of Pathology, University of Arizona, Tucson, Arizona; 3Institute of Human Genetics, Christian-Albrechts-University, Kiel, Germany

Introduction: Constitutive MHC class II expression is a hallmark of antigen-presenting cells and indispensable for antigen specific immune responses. It has been shown that certain B cell lymphoma entities evade immune recognition by downregulation of MHC molecules. We have previously identified recurrent chromosomal rearrangements of CIITA, the master regulator of MHC class II transcription, in primary mediastinal large B-cell lymphoma (PMBCL). Therefore, we aimed to explore the full spectrum, frequency and functional impact of CIITA alterations in a larger cohort of cases.

Methods: We analyzed PMBCL-derived cell lines and 45 PMBCL samples for the presence of mutations within the coding sequence (CDS) and the first 3kb of intron 1 using whole transcriptome paired-end sequencing (RNA-seq), deep amplicon and Sanger sequencing. We performed retroviral transductions of CIITA wild type and mutants and subsequently analyzed CIITA and HLA-DR expression using western blot and flow cytometry. Furthermore, we applied immunohistochemistry (IHC) to determine expression of CIITA and HLA-DR.

Results: All three cell lines showed biallelic CIITA aberrations consisting of missense mutations and structural genomic rearrangements. We found rearrangements in 15 of 41 primary cases (36.6%), 32% harboured CDS mutations, and in 21 cases (46.7%) we detected deletions and single nucleotide variants in intron 1. Genomic lesions in CIITA resulted in decreased CIITA protein, and in reduction of MHC class II surface expression. Further analyses revealed a significant correlation of decreased CIITA protein expression with the presence of CIITA rearrangements ($P$=0.025) and demonstrated a positive correlation between protein expression of CIITA and HLA-DR ($r$=0.413, $P$<0.0001).

Conclusions: We show that CIITA is frequently targeted by genomic rearrangements, CDS mutations and intronic deletions in PMBCL cell lines and clinical samples.

Outcome/Impact: Our study demonstrate that genomic alterations in CIITA contribute to downregulation of MHC class II in malignant lymphomas and therefore represent a potent mechanism of acquired immune privilege and escape from immune surveillance.
### 15. CONTRASTING EFFECTS ON HLA AND PD-L1 EXPRESSION THROUGH INHIBITION OF MAPK PATHWAY IN TRIPLE NEGATIVE BREAST CANCER CELLS

Rasmussen, Andrea¹, Ahmed Mostafa¹, Kensuke Hirasawa¹ and Sheila Drover¹

¹Division of BioMedical Sciences, Memorial University of Newfoundland

**Introduction:** Human leukocyte antigen (HLA) expression is required for anti-tumour T-cell immunity whereas programmed death ligand-1 (PD-L1) inhibits T-cell activation. The cytokine IFN-γ upregulates HLA and PD-L1, while the effects of the mitogen activated kinase pathway (MAPK) are variable. Our main objective was to determine if MEK-inhibition altered constitutive and IFN-γ-inducible expression of HLA and PD-L1 in a triple negative breast cancer cell (TNBC) line model.

**Methods:** MDA-MB-231, a TNBC line with hyper-activated MAPK, was treated or not with various MEK inhibitors in the presence and absence of IFN-γ. HLA-I, HLA-DR and PD-L1 were assessed using flow cytometry, immunoblotting and immunofluorescence.

**Results:** All inhibitors were shown to inhibit MEK activation. Two clinically relevant inhibitors, selumetinib and trametinib, decreased cell surface PD-L1, while maintaining or increasing HLA-I expression, suggesting that selective MEK inhibition could potentially augment the anti-tumour immune response in TNBC. PD0325901 and PD98059 had negligible effects on HLA and PD-L1, whereas U0126 profoundly decreased cell surface expression of both HLA and PD-L1. Increased pools of intracellular HLA-DR in U0126-treated cells suggested an ERK1/2-mediated trafficking mechanism, or a U0126 off-target effect.

**Conclusion:** Although all MEK inhibitors inactivated MEK1/2 and ERK1/2, they had variable effects on HLA and PD-L1. While U0126 decreased HLA and PD-L1, selumetinib and trametinib augmented HLA and importantly, decreased PD-L1 expression.

**Outcome/impact:** MAPK is frequently overactivated in TNBC, which has limited treatment options. Our results suggest that selumetinib and trametinib, currently in clinical trials for treating breast cancer, may also have immunotherapeutic applications by enhancing T-cell recognition of HLA-I/tumour peptides while reducing PD-L1 inhibition.

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16. THE EFFECTS OF HYPOXIA ON CENTROSOME FUNCTION IN PROSTATE CANCER

Taiakina, Daria¹; Laurence Pelletier²; Robert Bristow¹

¹Departments of Medical Biophysics and Radiation Oncology, University of Toronto; Ontario Cancer Institute; Princess Margaret Cancer Centre (University Health Network) ²Samuel Lunenfeld Research Institute, Mount Sinai Hospital; Department of Molecular Genetics, University of Toronto

Introduction: Hypoxia and centrosome aberrations are both prominent features of prostate cancer and have been shown to be markers of poor clinical prognosis. Our lab has previously shown that hypoxia down-regulates the expression and function of DNA damage repair (DDR) genes such as RAD51, BRCA1 and BRCA2; these proteins are also involved in centrosome function. We hypothesized that hypoxia causes centrosome aberrations in prostate cancer cells, as a potential mechanism of hypoxia-induced genomic instability.

Methods: DU145 prostate cancer cells were treated with hypoxia at 0.2% O₂ for 72 hours to induce deregulation of centrosome associated DDR genes. Centrosome aberrations were quantified in hypoxic and oxic cells, with and without re-oxygenation, with immunofluorescence (IF) labelling for centrosome markers PCNT, CETN and PCM1. Additionally, the expression of other centrosome-associated genes was quantified with qRT-PCR.

Results: Hypoxia treatment leads to an increase in centriolar satellites in mitotic DU145 cells. The phenotype was also observed in interphase in BPH1, U2OS and DU145 cells. Centriolar satellites were observed as extra CETN spots co-localizing to centriolar satellite marker PCM1, but not other centrosome markers. The induction in centriolar satellites was not re-oxygenation dependant. Moreover, hypoxia was shown to deregulate the expression of several centrosome associated genes, including PCM1 and PLK4.

Conclusions: Hypoxia induces centrosome aberrations and associated gene deregulation in a cell line and re-oxygenation independent manner.

Outcome/Impact: We are currently investigating if accumulation of centriolar satellites, which may act as assembly platforms for centrosomal proteins, leads to centrosome amplification. Centrosome amplification is a common feature of many cancers, and has been directly linked to chromosomal instability. If centrosome aberrations are specific to hypoxic cancer cells, centrosome and mitosis targeting drugs (such as PLK4 inhibitors) could provide a high therapeutic ratio and better outcome for patients with high hypoxic tumour fraction.
17. DEFINING MECHANISMS OF MIR-206-MEDIATED SUPPRESSION OF INVADOPODIA AND CANCER METASTASIS

Watt, Kathleen\(^1,2\), Arlidge Kip\(^2\), Truesdell Peter\(^1\), Renwick Neil,\(^3\) and Craig Andrew WB\(^1,2\)

\(^1\)Department of Cancer Biology and Genetics, Queen’s Cancer Research Institute; \(^2\)Department of Biomedical and Molecular Sciences, Queen’s University; \(^3\)Department of Pathology and Molecular Medicine, Queen’s University, Kingston, ON.

Introduction: Current treatments for metastatic cancers are limited and often ineffective, highlighting the desperate need for targeted therapies. Loss of tumour suppressor genes like p53 can lead to formation of extra-cellular matrix (ECM)-degrading invadopodia that promote metastasis. Recent studies have identified microRNA-206 (miR-206) as a tumour suppressor gene, with loss of miR-206 significantly associated with recurrence, metastasis, and reduced overall survival in multiple cancer types. Here, we tested the effects of miR-206 on invadopodia and tumour metastasis, and identified Transducer of Cdc42-dependent actin assembly-1 (Toca-1) as a novel target gene involved in cancer progression.

Methods: Expression of miR-206 was rescued using transient, stable, and inducible approaches in MDA-MB-231 breast cancer, A375 melanoma, and H1299 lung cancer cell models. Effects of miR-206 on invasion and motility were assessed using transwell invasion, and ECM degradation assays. Tumour growth and metastasis were examined using subcutaneous tumour xenograft assays using H1299 and A375 cells with stable and inducible miR-206 expression. Finally, Toca-1 was validated as a target of miR-206 by qRT-PCR and immunoblot.

Results: Upon restoring miR-206 expression in metastatic cancer cells, we observed suppression of Toca-1 expression at both the mRNA and protein levels, validating Toca-1 as a new target of miR-206. Further testing of miR-206 rescue cells revealed dramatic defects in ECM degradation and cell invasion in breast cancer and melanoma cell models. In tumour xenograft assays, stable rescue of miR-206 in H1299 tumours led to reduced tumour growth and a dramatic reduction in lung metastases. We are currently extending this to our melanoma models, and using our inducible system to define changes in metastasis-related targets of miR-206 that are aberrantly expressed in metastatic cancers.

Conclusions: Our study demonstrates a novel link between miR-206 and suppression of invadopodia formation via Toca-1 silencing, which likely contributes to the metastasis suppressing activity of miR-206 in multiple cancer types.

Impact: Identifying miR-206 target genes will promote discovery of metastasis drivers, and may yield new strategies to target this deadly stage of cancer progression.
18. SEQUENCE ANALYSIS OF CTDNA IN DIFFUSE LARGE B-CELL LYMPHOMA TO MONITOR TUMOUR PROGRESSION AND EVOLUTION

Yu, Stephen¹, Daniel Fornika¹, Miguel Alcaide³, Kristina Aluzaite¹, Koren Mann², Sarit Assouline², Nathalie Johnson², and Ryan Morin¹

¹Department of Molecular Biology and Biochemistry, Simon Fraser University; ²Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University

Introduction: Circulating tumour DNA (ctDNA) is an effective, non-invasive biomarker for monitoring tumour burden. Our study aims to detect and monitor ctDNA in individuals with diffuse large B-cell lymphoma (DLBCL) utilizing several sensitive sequencing-based methods.

Methods: We perform error-suppressed amplicon sequencing on plasma DNA, targeting mutated loci previously identified by whole exome (WES) or genome sequencing of tumours, to quantify the abundance of mutant alleles. To facilitate multiplex assessment of numerous loci per sample, we have adopted hybridization capture-based methods, which use hundreds of sequence-specific probes to enrich for regions commonly targeted by somatic point mutations and structural rearrangements and for a broader analysis, we employ probes targeting the entire exome.

Results: We analyzed 26 relapsed DLBCL patients for ctDNA, initially targeting a single somatic mutation in two plasma time points per patient. We detected ctDNA in 15 of 26 cases, with 70% of samples containing more than 50 mutant copies per mL of plasma. Of the 15 individuals, 11 showed fluctuations in ctDNA levels consistent with clinical response on trial. Possible causes for the discrepancies include clonal evolution and tumour heterogeneity. In a separate individual, we performed amplicon sequencing on six different plasma time points after relapse, targeting 17 somatic mutations identified by WES of tumour and plasma. We found the plasma exome was representative of mutations in the tumour and uncovered the presence of two genetically distinct tumour sub-clones at relapse. We have begun sequencing additional patient plasma samples using targeted capture and WES.

Conclusions: Our discrepancies suggest using single mutations for ctDNA analysis is insufficient to monitor tumour burden. Our capture-based assays profile ctDNA across many somatic mutations and structural rearrangements, giving a better estimate of tumour load and composition. Global sequencing of DNA from plasma, including WES, is enabling non-invasive tracking of clonal evolution in DLBCL patients.

Outcome/Impact: By sequencing serially collected DNA from plasma, we gain an improved understanding of the changes in somatic mutations that drive lymphoma progression during treatment and relapse.
19. PHYSICAL ACTIVITY BEHAVIOURS IN A SAMPLE OF OLDER BREAST AND PROSTATE CANCER SURVIVORS IN NEWFOUNDLAND, CANADA

Buote, Richard¹; Jonathan Greenland²; Kevin Power¹; David Behm¹; Erin McGowan¹

¹School of Human Kinetics and Recreation, MUN, Newfoundland; ²Eastern Health of Newfoundland

Introduction: Older cancer survivors experience the combined negative effects that accompany both aging and a cancer diagnosis. Physical activity (PA) has been found to be a beneficial strategy for improving cancer survivorship. The purpose of this study was to characterize PA behaviours in a sample of older (60+) breast and prostate cancer survivors in Newfoundland (NL), Canada.

Methods: Older breast and prostate cancer survivors were recruited from local support groups, advertisements, and the NL Cancer registry to complete a mailed questionnaire (53.2% response rate).

Results: Of the 58 returned questionnaires (mean age 70 ± 5.1; 53% breast cancer survivors), only 30% reported meeting the CSEP PA recommendations of 150 moderate-vigorous minutes of activity weekly. Prostate cancer survivors reported more activity than breast cancer survivors (33% vs 27%), however this difference was not statistically significant. Although many did not meet recommended guidelines, almost half (48%) reported walking at least 100 minutes weekly, typically at a casual pace (59%). Only 21% and 22% of survivors reported engaging in sports or strength training, respectively. Additionally, only 36% reported having someone involved with their cancer care discuss PA with them. These findings are in contrast to respondent’s attitudes and beliefs with most reporting they believed PA was enjoyable (79%), important (86%), and beneficial (81%).

Conclusion: The majority of our sample was inactive, however their attitudes and beliefs demonstrate that they value and recognize the benefits of PA. Therefore, based on our results it seems that older prostate and breast cancer survivors may be a receptive audience for targeted PA behaviour change interventions.

Outcome/Impact: Despite being a disease of age, little research has explored cancer survivorship in older adults. This is unfortunate, as older cancer survivors experience poorer QoL, physical functioning, and general health compared to individuals with no cancer history, and have an increased risk of developing recurrent and secondary cancers, and other chronic diseases. PA has been consistently shown to improve QoL and disease outcomes in cancer survivors. Therefore, it is important to develop and implement novel and innovative methods for increasing PA in older cancer survivors to improve survivorship.
20. DETECTION OF GENOMIC REARRANGEMENTS IN ARCHIVAL LYMPHOMA TISSUES USING TARGETED CAPTURE SEQUENCING

Chong, Lauren C.¹,², Ben-Neriah Susana¹, Twain David D. W.¹,³, Mottok Anja¹,³, Chan Fong Chun¹,², Zhao Yongjun⁴, Shah Sohrab P.³, Marra Marco A.¹,⁴, Scott David W.¹, Gascoyne Randy G.¹,³, Mungall Andrew J.⁴, Steidl Christian¹,³

¹Centre for Lymphoid Cancer, BC Cancer Agency, Vancouver, BC, Canada; ²Bioinformatics Training Program, ³Department of Pathology and Laboratory Medicine, UBC, Vancouver, BC, Canada; ⁴Genome Sciences Centre, Vancouver, BC, Canada

Introduction: Genomic rearrangements are characteristic of non-Hodgkin lymphoma (NHL) pathogenesis, and recurrent rearrangements involving the MHC class II transactivator CIITA and program death ligands PDL1 and PDL2 have been shown to contribute to immune privilege with implications for novel therapeutic approaches. However, the landscape of fusion partners for these genes has not been well characterized and methods that utilize formalin-fixed paraffin-embedded (FFPE) tumour samples for breakpoint discovery have not been explored.

Methods: 57 NHL patients with CIITA or PDL1/2 rearrangements determined by fluorescence in situ hybridization (FISH) break-apart assays were selected. DNA was captured from FFPE tumour samples using a custom Agilent SureSelect design surrounding CIITA and PDL1/2 and sequenced on an Illumina HiSeq 2500. Reads were aligned to the human reference and multiple structural variant (SV) detection tools were used in an ensemble approach to generate predictions. Assembly-based SV detection was also utilized.

Results: 41 samples (72%) had one or more predicted SVs involving CIITA or PDL1/2, and 31 (54%) contained events matching observed FISH patterns. 26 interchromosomal translocations were detected, some involving known recurrently rearranged genes (e.g., IGH) and others involving genes not previously implicated in NHL rearrangements (e.g., CD44). In addition, many FISH-silent intrachromosomal SVs were predicted. PCR and Sanger sequencing was performed for 12 variants (8 translocations; 3 deletions; 1 duplication) and all successfully validated.

Conclusions: A capture-based methodology for FFPE tissue can identify unknown fusion partners and describe breakpoint anatomy at base-pair resolution. While FISH-based assays appear to have increased sensitivity for large-scale rearrangements, the capture approach provides improved resolution and ability to detect smaller SVs.

Outcome/Impact: Our pipeline is a valuable research tool in conjunction with FISH to explore the full spectrum of rearrangements in NHL subtypes. In future studies we will capture a wider range of known rearrangement hotspots and assess if our method has clinical utility for informing on treatment strategies and decision-making.
21. IDENTIFYING NOVEL REGULATION MECHANISMS OF INNATE VIRAL RESPONSE

Davis, Colin1,2, Rachel McPhedran1,2, Parisa Mazrooei3,4, John Bell1,2, Nicole Forbes1,2, Fabrice Le Boeuf,1,2 Mathieu Lupien3,4, Jean-Simon Diallo1,2

1Center for Innovative Cancer Research, Ottawa Hospital Research Institute, Ottawa, ON, Canada; 2Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada; 3Ontario Cancer Institute, Princess Margaret Cancer Center/University Health Network, Toronto, ON, Canada; 4Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

Introduction: Investigating the source of hypersensitivity to infection in the CT26.LacZ cancer cell line that leads to their successful and reproducible treatment with oncolytic viruses (OVs). Determining key biological pathway components leading to the hypersensitive phenotype to guide the development of new targets for viral sensitizers.

Methods: Top-down analysis using large scale genetic (microarray) and epigenetic (ChIP-seq) comparisons of CT26.LacZ to their OV resistant parental cell line CT26.WT.

Results: Both the microarray analysis and ChIP-seq transcription factor binding site results converge on the same finding that genes downstream of response to interferon-beta (Innate immunity signaling cytokine that alerts neighboring cells of viral infection) fail to be activated in the hypersensitive CT26.LacZ cell line. Follow-up in vitro studies confirmed that while the hypersensitive cells do produce interferon-beta, they do not response to its presence and fail to become protected against viral infection. As CT26.LacZ have been modified to express the LacZ gene, mutational analysis was performed and identified 4 integration sites with 2 in intronic regions of transcribed genes which have not previously been associated as effectors of interferon response.

Conclusions: In the ongoing study we are following up on the 2 candidate genes through knockdown and over-expression to reproduce the hypersensitive phenotype. Once the target is confirmed we will begin the development of new VSEs that specifically target the gene of interest.

Outcome / Impact: Oncolytic viruses are very effective when patients have OV sensitive tumours. However, some patients or even subregions of a patients tumours can be resistant to viral infection and result in failed treatment or relapse. With VSE pretreatment we hope to extend OV availability to patients with resistant tumours and furthermore to ensure that no relapse of resistant tumour subregions occur.
22. GENES ASSOCIATED WITH THE REVERSE-WARBURG EFFECT (RWE) AS PROGNOSTIC BIOMARKERS IN PROSTATE ADENOCARCINOMA

Georgescu, I.¹, Gooding RJ², Park PC¹

¹Department of Pathology and Molecular Medicine, Queen’s University; ²Department of Physics, Queen’s University

Introduction: Prostate biopsies frequently fail to sample clinically relevant high-grade tumour foci, leading to underestimation of the risk of disease progression in 30% of cases. In several aggressive cancer types, the tumour microenvironment exhibits an altered, pro-tumourigenic metabolic phenotype consistent with Reverse Warburg Effect (RWE). We hypothesize that RWE-associated gene signatures can be used to distinguish between the stroma adjacent to Gleason pattern (GP) 3 and 4 cancer foci.

Methods: A targeted panel of 101 RWE-associated genes was identified using a meta-analysis of publicly available gene expression data, pathway analysis and literature mining. A discovery cohort of 60 FFPE radical prostatectomy samples, evenly divided between Gleason Score (GS) 3+3 and GS ≥4+3, was identified. mRNA was then extracted from laser-capture microdissected stromal regions adjacent to either GP3 or GP4 foci, and subjected to targeted gene expression analysis using Nanostring nCounter technology. Normalization and univariate gene analysis were conducted using RStudio.

Results: Eight genes were determined to be significantly differentially expressed between the stroma surrounding GP 3 and GP4 across multiple statistical tests. The genes (p-value, log2 fold changes (FC) GP4 vs. GP3) are listed as follows: ACTA2 (p = 0.022, FC=1.29) COL1A2 (p = 0.008, FC=2.34), CTGF (p = 0.037, FC=1.08) FOXO1 (p = 0.015, FC = -2.38), HK2 (p = 0.045, FC = -1.04), MAP1LC3B (p = 0.01, FC = -2.71), PDGFRB (p = 0.044, FC = 2.70), and SPARC (p = 0.009, FC = 2.35). FOXO1 was found to be the single best predictor of the presence of GP4 with a ROC-AUC of 0.82 (p = 0.005).

Conclusions: Results show that the stroma associated with GP4 cancer foci have distinct metabolic signatures, compared to GP3 cancer foci. Future work will employ regression analysis to develop a multivariate gene signature capable of distinguishing between GP3 and GP4, and immunohistochemical analysis will be used to quantify the spatial extent of the RWE influence surrounding GP4 foci.

Outcome/Impact: Stroma-based biomarkers that can predict the presence of high-grade, high-risk tumour foci can effectively increase the target size for biopsy sampling, and thus increase the sensitivity and accuracy of risk stratification in prostate cancer.
23. PPARγ LOSS INCREASES METASTATIC POTENTIAL OF HER2+ BREAST TUMOURS IN MAMMARY EPITHELIAL TARGETED KNOCKOUT MICE

Lightbody, Elizabeth D.¹, Rubino Rachel E.², Apostoli Anthony J.², Skelhorne-Gross Graham², Schneider Mark M.¹, SenGupta Sandip K.¹, and Nicol Christopher JB.¹-³

¹Departments of Pathology and Molecular Medicine; ²Cancer Biology and Genetics, Cancer Research Institute; ³Biomedical and Molecular Sciences, Queen’s University, Kingston, ON

Introduction: Breast tumours that overexpress epidermal growth factor receptor 2 (HER2+) are associated with a poor patient prognosis compared to HER2- tumours. Peroxisome proliferator-activated receptor (PPARγ) is a transcription factor that regulates the expression of genes involved in sugar and fat metabolism. Interestingly, PPARγ-activating drugs decrease human breast cancer cell growth in vitro by inhibiting activation of HER2 family members, although the impact of PPARγ signaling on HER2 signaling and patient survival is still unresolved.

Methods: We interbred a spontaneous HER2 overexpressing breast tumour mouse model, MMTV-Neu-IRES-Cre (NIC) with our PPARγ-floxed mice (PPARγ-MG KO) to create a unique model (PPARγ-NIC KO). This in vivo model has targeted PPARγ deletion in the same HER2+ transformed mammary epithelial cells that drive breast tumourigenesis. Western blot analysis and immunofluorescence was used to evaluate PPARγ and HER2 expression and localization in mouse tumours. We also established a mouse tumourigenic cell line from a PPARγ-NIC KO lung metastatic tumour (PPARγ-NIC KOmet) to define in vitro signaling interactions.

Results: PPARγ expression was lost as tumours progressed from primary mammary tumours to lung metastatic tumours, and resulted in an increase of phosphoHER2+ protein (pHER2+) levels at phosphorylation site 877. The PPARγ-NIC KO primary and metastatic tumours had significantly higher automated immunofluorescence HER2 H-Scores compared to PPARγ-WT and 7,12-dimethylbenz[a]anthracene (DMBA) chemically-induced mammary tumours. The PPARγ-NIC KOmet cell line characterized by western blot lacked PPARγ and express similar pHER2/total HER2 levels as the original lung metastatic tumour from which it was derived.

Conclusions: These preliminary results suggest that a loss of PPARγ expression greatly enhances the metastatic spread of HER2/neu+ mammary tumours to the lung.

Outcome/Impact: This project will definitively confirm PPARγ and HER2 signaling interactions that impact the metastatic potential of HER2+ breast tumours. This project will also unveil novel PPARγ upstream and downstream targets that may be used as predictive biomarkers for HER2+ breast tumour patients susceptible to increased metastasis, and lay a foundation for similar studies in other human breast tumour subtypes.
24. EPIGENETIC LANDSCAPES OF PRIMARY ACUTE MYELOID LEUKEMIC BLAST CELLS

Lorzadeh, A.1,2, Parker J.2, Knapp D.3,4, Bilenky M.1,2, Moksa M.1, Paliouras G.2, Tam F.1, Hogge D.4,5, Marra M.A.2,3, Eaves C.3,4,5, Karsan A.2,5, Hirst M.1,2

1Department of Microbiology and Immunology, UBC; 2Canada’s Michael Smith Genome Science Centre, BCCA; 3Department of Medical Genetics, UBC; 4Terry Fox Laboratory, BCCA; 5Department of Pathology and Laboratory Medicine, UBC

Introduction: Neomorphic isocitrate dehydrogenase (IDH) mutations are recurrent genetic lesions observed in Acute Myeloid Leukemia (AML) that drive the accumulation of the metabolite D-2-hydroxyglutarate (D2-HG). D2-HG is an inhibitor of 2-oxoglutarate-dependent dioxygenases (2-OGDD), a family of enzymes that include a class of epigenetic modifiers that are responsible for histone and DNA demethylation. In mouse models neomorphic IDH mutations have been shown to reversibly transform progenitor hematopoietic populations harboring FMS-like tyrosine kinase 3 internal tandem duplications (FLT3ITD). To investigate the role D2-HG might play in remodelling the epigenome of primary FLT3ITD AML blast cells we performed native ChIP-seq on cord blood derived CD34+ cells and primary AML cells harboring FLT3ITD in the presence and absence of neomorphic IDH mutations.

Methods: We applied an optimized native ChIP-seq protocol to profile five histone modifications (histone3 lysine 4 tri-methylation (H3K4me3), H3K4me1, H3K27me3, H3K36me3, and H3K9me3). ChIP-seq was conducted on aliquots of ~10,000 CD34+ cells purified from cord blood by a 2-step Rosette-Sep-EasySep procedure to ≥95% purity and ~10,000 AML cells harboring the FLT3ITD allele and IDH2R140Q or IDH2WT alleles. The resulting libraries prepared from these cells were sequenced on an Illumina HiSeq platform in paired-end mode, analyzed using our ChIP-seq analysis tool FindER and integrated with existing matched RNA-seq datasets.

Results: Pair-wise comparisons between AML-IDH2WT-FLT3ITD and AML-IDH2R140Q-FLT3ITD revealed a global increase in H3K27me3 (a repressive histone modification) in AML-IDH2R140Q-FLT3ITD compared to that of AML-IDH2WT-FLT3ITD. Among genomic regions with differentially modified nucleosomes were the HOXA and HOXB clusters that include the key AML driver HOXA9. Matched RNA-seq datasets confirmed transcriptional repression of genes with H3K27me3 gains in their promoter. Overall our results are consistent with a model where inhibition of 2-OGDD histone demethylases, such as the UTX (H3K27me3 demethylase) by 2D-HG lead localized gains of H3K27me3 and gene repression.

Conclusions: Our results support a model where IDH neomorphic mutations lead to localized reprogramming of the epigenetic landscape of primary AML through the inhibition 2-OGDD histone demethylases.
25. THE STATUS OF P53 AND RB AND THE OUTCOME OF PROSTATE CANCER

Mahamud, O.1, Lalonde E1,4, Zafarana G3, Sykes J3, Boutros PC1,5, Bristow RG1,2,3

Departments of 1Medical Biophysics; 2Radiation Oncology, University of Toronto; 3Radiation Medicine Program, Princess Margaret Cancer Centre, University Health Network; 4Informatics & Biocomputing Platform, Ontario Institute for Cancer Research

Introduction: Prostate cancer (CaP) is the most commonly diagnosed cancer among Canadian men (24,000 men/year), with over 4000 men dying of the disease each year. To help guide treatment, clinicians use local T-stage, prostate specific antigen (PSA) and the pathologic Gleason score to place men with localized CaP into low, intermediate and high-risk groups for CaP-specific mortality. Unfortunately, current clinical prognostic factors only explain a moderate proportion of the observed heterogeneity in clinical outcome. With 20-60% of localized CaP patients failing primary local treatment, we urgently need better predictors of individual prognosis and treatment response to triage patients into customized CaP treatment regimes.

Methods: Using array comparative genomic hybridization (aCGH), we characterized copy number alterations (CNAs) in biopsies derived from 126 patients who underwent image-guided radiotherapy (IGRT). The IGRT cohort was compared to a radical prostatectomy cohort of 154 patients (MSKCC). CNAs were then tested for their independent prognostic capability using Kaplan-Meir and Cox proportional hazard models.

Results: Patients with allelic loss of p53 and Rb loci exhibited increased genomic instability (P=0.001 and P< 0.0001). Tumours that presented either a p53 or Rb loss had increased biochemical failure at 5 years (p53: bRFR 76% vs 43%, HR: 3.17 P = 0.00088 Rb: bRFR 78% vs 56%, HR: 1.86, P = 0.023). Patients with combined allelic loss in p53 and Rb had a poor prognosis when compared to patients with normal p53 and Rb status (bRFR 81% vs 40% P = 0.00017). On a multivariate analysis, only p53 loss was a significant independent predictor after correcting for PSA, GS, and T-category (HR = 3.53, P = 0.00067).

Conclusions: Irrespective of treatment modality (RadP vs IGRT) p53 loss is a significant prognostic indicator of PCa failure.

Outcome/Impact: Triaging patients by the use of CNAs within pre-treatment biopsies may allow for better use of systemic therapies to target sub-clinical metastases or locally recurrent disease and improve clinical outcomes.
26. IDENTIFICATION OF GENES INVOLVED IN BREAST CANCER RESPONSE TO PACLITAXEL USING A TOTAL GENOME KNOCKDOWN SCREEN

Sultan, M.¹, ML Thomas¹, KM Coyle¹, TT Huynh¹,², CA Giacomantonio¹,³, P Marcato¹

Departments of Pathology¹, Microbiology and Immunology² and Surgery³, Dalhousie University, Halifax, NS, Canada

Introduction: Despite advances in detection methods and therapy, one in every twenty seven women will die from breast cancer. In addition to surgery and radiation, treatment often involves the use of chemotherapeutics including taxanes such as paclitaxel. Unfortunately, some patients do not respond to this drug. Thus being able to identify the genes which when expressed in a tumour predict sensitivity or resistance to treatment prior to administration of paclitaxel would improve treatment efficacy and patient survival.

Methods: A genome-wide lentiviral-based shRNA screen was performed with MDA-MB-231 tumour xenografts in female NOD/SCID mice. Groups of six mice received daily intraperitoneal injections of paclitaxel (10 mg/kg) or phosphate buffered saline. Treatment started 24 days post cancer cell injection and continued for eight days. Subsequently, all mice were sacrificed, the tumours harvested, genomic DNA extracted and shRNA sequences retrieved. This allowed the identification of enriched and depleted shRNA sequences that theoretically target paclitaxel sensitivity and resistance genes, respectively.

Results: Completion of 6 replicates of the total genome shRNA screen identified 26 putative paclitaxel sensitivity genes and 14 putative paclitaxel resistance genes for breast cancer. Some of the genes identified in the screen have never been associated with paclitaxel sensitivity or resistance before and potentially represent novel findings. However, some of the genes have been associated with metabolic pathways targeted by paclitaxel or previously linked to chemotherapy resistance and metastasis in breast cancer and other cancers.

Conclusions: Using a total genome knockdown screen, we have identified several genes with a potential novel role in taxane sensitivity and resistance in breast cancer.

Outcome/Impact: Confirmation experiments will help generate a genetic profile which can be used to identify candidate breast cancer patients who would most benefit from paclitaxel treatment as opposed to treatment with other drugs. Additionally, some of the identified genes are potentially novel drug targets.
27. DYNAMIC REGULATION OF CD24 EXPRESSION AND RELEASE OF CD24-CONTAINING MICROVESICLES IN IMMATURE B CELLS IN RESPONSE TO CD24 ENGAGEMENT

Ayre, Craig D.¹, Marcus Elstner, Emily S. Moores and Sherri L. Christian*.

Department of Biochemistry, Memorial University of Newfoundland, St. John’s, NL

Introduction: The glycophasphatidylinositol (GPI)-anchored cell surface receptor CD24 (also called heat stable antigen) promotes the apoptosis of progenitor and precursor B-lymphocytes. However, the immediate proximal events that occur after engagement of CD24 in B cells are not precisely understood. The regulation apoptosis in B cell development is critical in the prevention of lymphoproliferative disorders, thus identifying the role CD24 plays in regulating this process may improve our understanding of these disease processes.

Methods: We performed a bioinformatics analysis of mouse (Mus musculus) gene expression data from the Immunological Genome Project to identify potential CD24 signalling partners or effectors based on the guilt by association theory. Subsequently we validated the mouse WEHI-231 B cell line as a model of CD24-mediated apoptosis using flow cytometry. Using both flow cytometry and transmission electron microscopy, we also examined CD24 protein expression and localization in response to antibody-mediated stimulation.

Results: We found that known vesicle trafficking and cellular organization genes have similar expression patterns to CD24 during B cell development in the bone marrow. We thus hypothesized that CD24 regulates vesicle trafficking. We next found that CD24 surface protein expression is rapidly and dynamically regulated in both primary immature B cells and WEHI-231 cells in response to engagement of CD24. The change in surface expression was not mediated by classical endocytosis or exocytosis but through the release of plasma membrane-derived extracellular microvesicles (EMV). Furthermore, in response to CD24 engagement we observed a clear exchange of CD24 between different populations of B cells.

Conclusions: We show that engagement of CD24 in immature B cells results in a dynamic regulation of surface CD24 protein and a redistribution of CD24 within the population.

Outcome/Impact: Identifying how CD24 functions may increase our understanding of B cell development and provide insight into B cell lymphoproliferative diseases such as leukemia. The role of CD24 in promoting EMV formation represents a novel avenue in understanding how B cell apoptosis may be controlled. Identifying the molecular partners and effectors of CD24 may uncover new therapeutic targets, which may be exploited in the management of these diseases.
28. DEFINING MOLECULAR MECHANISMS LINKING ENDOPHILIN A2 TO METASTASIS IN HUMAN BREAST CANCER MODELS

Baldassarre, Tomas¹², Watt Kathleen¹², Truesdell Peter¹², Schneider Mark³, Sengupta Sandip³, and Craig Andrew WB¹²

¹Department of Biomedical and Molecular Sciences; ²Cancer Biology & Genetics Division, Queen’s Cancer Research Institute; ³Department of Pathology & Molecular Medicine, Queen’s University, Kingston, Ontario, Canada

Introduction: Breast cancers in human epidermal growth factor receptor 2 (HER2) and triple-negative breast cancer (TNBC) subtypes have high rates of tumour metastasis. This is driven by cells undergoing Epithelial-Mesenchymal Transition (EMT), and subsequent formation of invadopodia that degrade extracellular matrix (ECM) in basement membranes to spread locally and colonize distant sites. Endophilin A2 (Endo II) is an adaptor protein that coordinates internalization and trafficking of receptors that drive invadopodia formation and breast cancer metastasis. We have recently identified Endo II as a positive regulator of human TNBC metastasis, invadopodia formation, Membrane-Type 1 Matrix Metalloproteinase (MT1-MMP) endocytosis, and Epidermal Growth Factor Receptor (EGFR) internalization and signaling. Here, we extend these studies to HER2 breast cancers, and define molecular mechanisms that link Endo II to EMT, invadopodia formation and cell invasion.

Methods: Endo II expression was profiled by immunohistochemistry in human breast tumour tissue microarrays. Stable silencing of Endo II was achieved using lentiviral shRNAs to knockdown (KD) Endo II in HER2 and TNBC cell lines. Confocal and TIRF microscopy were used to assess effects of Endo II on MT1-MMP trafficking in TNBC cells. Mammary orthotopic xenograft assays were performed to test effects of Endo II KD on EMT and tumour metastasis.

Results: Analysis of Endo II expression in human invasive ductal carcinomas revealed significantly higher expression of Endo II in HER2 primary tumours, and paired lymph node metastases. High levels of Endo II mRNA were associated with reduced rates of relapse free survival in patients with lymph node positive, estrogen receptor negative tumours. Mechanistically, this may be explained by reduced EGFR signaling, inefficient MT1-MMP internalization and trafficking, and impaired EMT in Endo II KD cells. These results are extended to mammary orthotopic tumour xenograft assays to further dissect the contributions of Endo II to metastatic tumour progression.

Conclusions and Impact: These findings identify Endo II as a potential poor prognosis biomarker in HER2 and TNBC. This research could open new avenues for targeted therapy focusing on endocytosis of receptors that drive metastasis.
29. REOVIRUS-DRIVEN CD11B⁺, GR-1⁺, LY6CʰIGH MDSCS AUGMENT TUMOUR-ASSOCIATED IMMUNOSUPPRESSION IMMEDIATELY FOLLOWING ITS THERAPEUTIC ADMINISTRATION

Clements, Derek R.¹, Andra M Sterea², Youra Kim¹, Erin Helson³, Anna Nunokawa³, Shashi A Gujar³,⁴,⁵ and Patrick WK Lee¹,³,⁵

Departments of ¹Pathology, ²Biology, ³Microbiology & Immunology, Dalhousie University, Halifax, NS, Canada; ⁴Strategy & Organizational Performance, IWK Health Centre, Halifax, NS, Canada. ⁵Co-corresponding authors.

Introduction: Reovirus-based anti-cancer therapy (oncotherapy) directly kills cancer cells, over turns tumour-associated immune evasion mechanisms and promotes anti-tumour immunity. However, recent studies illustrate that reovirus also induces the accumulation of immunosuppressive cells (myeloid-derived suppressor cells [MDSCs]) during the early stages of oncotherapy. Thus, this study focuses on comprehensively examining the phenotypic heterogeneity, frequencies of myeloid cells, functions, and migratory potential of the tumour milieu throughout the course of oncotherapy.

Methods: Mouse ovarian surface epithelial tumour-bearing C57BL/6 mice were treated with a therapeutic regimen of reovirus and the frequencies of myeloid cells and various immunological parameters (using chemotactic assays, cytokine arrays, qPCR, and T-cell suppression assays were examined throughout reovirus therapy.

Results: Our in vitro data shows that reovirus can induce the preferential differentiation of CD11b⁺, Gr-1⁺, Ly6Cʰigh myeloid cells from bone marrow progenitor cells. Furthermore, reovirus administration in tumour-bearing hosts drives time-dependent recruitment of CD11b⁺, Gr-1⁺, Ly6Cʰigh myeloid cells in the tumour milieu, which is further supported by virus-induced increased expression of numerous immune factors involved in MDSC survival and trafficking. Most importantly, CD11b⁺, Gr-1⁺, Ly6Cʰigh myeloid cells specifically potentiate the suppression of T cell proliferation, cytotoxicity, and are associated with the absence of IFN-γ response in the tumour microenvironment early during oncotherapy.

Conclusion: Contrary to this existing paradigm, we demonstrate that reovirus augments tumour-associated immunosuppression immediately following its therapeutic administration through the recruitment of highly suppressive CD11b⁺, Gr-1⁺, Ly6Cʰigh MDSCs. Considering that the qualitative traits of a specific anti-tumour immunity are largely dictated by the immunological events that precede its development, our findings are of critical importance and must be considered while devising complementary immune interventions aimed at enhancing the efficacy of oncolytic virus-based anti-cancer immunotherapies.

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30. **PZ-DHA, A NOVEL POLYPHENOL FATTY ACID ESTER DERIVATIVE, IS CYTOTOXIC FOR BREAST CANCER CELLS**

**Fernando, Wasundara**¹, David W. Hoskin¹²³ and H. P. Vasantha Rupasinghe¹⁴

Departments of Pathology¹, Microbiology and Immunology² and Surgery³, Faculty of Medicine, Dalhousie University, Halifax, NS, Canada; Department of Environmental Sciences⁴, Faculty of Agriculture, Dalhousie University, Truro, NS, Canada

**Introduction:** Polyphenols demonstrate tremendous disease-fighting properties against chronic disorders; however, diminished bioavailability of some polyphenols is a major restriction. We, therefore, combined phloridzin (PZ), a dihydrochalcone found in apple peels, with docosahexaenoic acid (DHA), an omega-3 fatty acid, to increase lipophilicity. In the current study, we evaluated the effect of phloridzin docosahexaenoate (PZ-DHA) on breast cancer (BC) cell growth.

**Method:** The *in vitro* cytotoxic properties of PZ-DHA against MDA-MB-231, MDA-MB-468, 4T-1, MCF-7 and T-47D BC cells and human mammary epithelial cells (HMECs) were tested using MTS and acid phosphatase assays. Cell death was confirmed using 7-AAD staining and apoptosis induction was studied using caspase 3/7 activation and DNA fragmentation (TUNEL) assays. PZ-DHA-induced H₂O₂ production in HCO₃⁻ containing medium was evaluated using an Amplex red assay. Involvement of reactive oxygen species (ROS) in cell death was assessed using Annexin-V-FLUOS/propidium iodide (PI) staining of PZ-DHA treated cells incubated in the presence or absence of N-acetyl cysteine. *In vivo* tumour suppression by PZ-DHA was examined by intra-tumoural injection of PZ-DHA to MDA-MB-231 tumour bearing non-obese diabetic severe combined immunodeficient (NOD-SCID) female mice. Tumours were then excised and stained with haematoxylin and eosin. For comparison, PZ and DHA were included in all the experiments. Statistical analysis was performed at α=0.05/0.01/0.001.

**Results:** All the PZ-DHA-treated BC cells showed a time- and dose-dependent reduction in viable cell number with no toxic effects to HMECs at low doses. PZ-DHA induced caspase 3/7 and DNA fragmentation but did not cause ROS production. MDA-MB-231 xenograft growth in NOD-SCID mice was suppressed significantly by PZ-DHA with no adverse side effects.

**Conclusions:** PZ-DHA suppressed the growth of both triple negative and estrogen receptor-positive BC cells *in vitro* through a ROS-independent mechanism. PZ-DHA also suppressed MDA-MB-231 tumour growth *in vivo*.

**Outcome/Impact:** PZ-DHA shows potential as an anticancer agent against BC. We are currently studying the pharmacokinetics of PZ-DHA, which may allow us to enhance its anticancer properties.
31. IL4R MUTATIONS IN PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA

Gunawardana, Jay1,2, Tessa VanTol1, Katina Mak1, David Twa1,2, Elizabeth Chavez1, Bruce Woolcock1, Anja Mottok1,2, Shannon Healy1, Adèle Telenius1, Susana Ben-Neriah1, Hennady Shulha1, Stacy Hung1, Robert Kridel1,2, Randy Gascoyne1,2 and Christian Steidl1,2.

1Lymphoid Cancer Research, BC Cancer Agency; 2Pathology and Laboratory Medicine, UBC

Introduction: Primary mediastinal B cell lymphoma (PMBCL) is a subtype of aggressive B cell lymphoma that characteristically presents as a mass in the anterior mediastinum. Some PMBCL patients suffer from refractory disease and salvage therapies are often ineffective. Development of targeted therapies is impeded by the lack of knowledge about the mutational landscape in the lymphoma genomes and mutation-associated phenotypes. We recently reported somatic mutations in the transcriptome of 10 PMBCL cases and found a hotspot mutation in IL4R. Activation of IL4R by IL4 and/or IL13 initiates intracellular signaling mediated by the phosphorylation of JAnus Kinase-Signal Transducer and Activation of Transcription (JAK-STAT) pathway. We hypothesize that constitutively active JAK-STAT in PMBCL is in part due to gain-of-function IL4R mutations signaling through this oncogenic pathway.

Methods: Lymph node biopsies from 62 PMBCL cases were selected from the BCCA tissue archive. DNA from PMBCL tumours were extracted for IL4R exonic PCR amplification and high-throughput sequencing. WT and mutant (I242N) IL4R were expressed in the lymphoma cell line DEV and in HEK-293 cells expressing STAT6. Supernatant from cultured cells were used to measure the activity of STAT6-dependent expression of secreted embryonic alkaline phosphatase (SEAP). Extracted RNA and protein from transfected cells were used for qRT-PCR and Western blotting.

Results: Somatic IL4R mutations were found in 18 of 65 (28%) cases confirming a hotspot mutation (I242N) in 11 of 18 (61%) mutated cases. Ectopic expression of the mutant in HEK-293 cells showed increased SEAP levels compared to WT indicating STAT6 activation independent of cytokine stimulation. Introduction of the mutant into DEV cells showed IL4 independent hyperphosphorylation of JAK2, STAT5 and STAT6 proteins and upregulation of the B cell activation marker CD23.

Conclusions: IL4R is frequently mutated in PMBCL with a recurrent hotspot affecting the transmembrane domain of the protein. Functional analyses characterize these mutations as gain-of-function leading to constitutive activation of the JAK-STAT pathway.

Outcome/Impact: These data suggest IL4R mutations as novel driver alterations in PMBCL and provide a rational therapeutic target.
32. THE PGC-1α/ERRα AXIS REPresses FOLate METABOLISM AND PROMotes SENSITIVITY TO ANTI-FOLate THERAPY IN BREAST CANCER

Papadopoli, David J.1,2*; Audet-Walsh, Étienne2*; Yee, Tracey1,2; Giguère, Vincent1,2,3; St-Pierre, Julie1,2

1Department of Biochemistry, 2Rosalind and Morris Goodman Cancer Research Centre, 3Departments of Medicine and Oncology, McGill University, Montreal, QC, Canada
*Equal contribution

Introduction: Cancer cells display an altered metabolism, and the identification of the molecular players orchestrating metabolic changes is an intense area of cancer research. The main aim of our work is to elucidate the specific metabolic gene networks dependent on AMPK (AMP-activated protein kinase) / PGC-1 (peroxisome-proliferator activated receptor γ coactivator-1) / ERR (estrogen-related receptor) signalling, and how their activity influences breast tumour growth.

Methods: The impact of AMPK/PGC-1α/ERRα signalling on the metabolic state of HER2+ breast cancer was determined using a combination of genomics and metabolomics. We performed gain and loss of functions experiments for PGC-1α and ERRα to determine their contribution to the gene expression and metabolite profiles.

Results: The intersection of gene expression and promoter binding datasets upon AMPK activation in breast cancer cells showed that activation of AMPK significantly increased the expression of PGC-1α/ERRα and promoted the binding of ERRα to its cognate sites. Also, these data revealed that PGC-1α/ERRα binds and negatively regulates the expression of folate cycle genes, resulting in perturbations of metabolic pathways associated with the folate cycle, such as de-novo purine biosynthesis. The clinical relevance of these data is illustrated by the fact that the PGC-1α/ERRα mediated regulation of folate metabolism plays a central role in the response of breast cancer cells to methotrexate treatment both in vitro and in vivo.

Conclusions: Together, these data demonstrate that PGC-1α/ERRα act as central repressors of folate cycle metabolism and sensitize cells to methotrexate treatment.

Outcome/Impact: AMPK, PGC-1α, and ERRα are well-established metabolic regulators in cells. This study reveals the importance of the AMPK/PGC-1α/ERRα signalling pathway in controlling folate metabolism and highlights their role as novel modulators of response to anti-folate therapy in breast cancer.
33. MET AND FGFR1 CO-OPERATE TO PROMOTE TUMOUR INITIATION AND PROGRESSION IN A MODEL OF CLAUDIN-LOW BREAST CANCER

Sung, Vanessa Y.C.1,2, Knight Jennifer F.1,2, Saleh Sadiq M.1,2, Savage Paul1,3, Monast Anie1,2, Park Morag1,2

1Goodman Cancer Research Centre, McGill University, Montreal; Departments of 2Biochemistry, 3Experimental Medicine, McGill University, Montreal

Introduction: In breast cancer, elevated levels of the MET receptor are correlated with triple negative breast cancer (TNBC) subtypes and poor patient outcome, but reasons for this are not well understood. Here, we investigate the role of MET signaling and its cooperating pathways in TNBC.

Methods: We previously generated a transgenic mouse model in which expression of mutant Met is combined with conditional deletion of Trp53 in the mammary gland (Met\textsuperscript{mt};Trp53fl/+). To assess tumour-initiating potential, we utilized the well-established sphere-forming assay, which enriches for tumour-initiating cells (TIC) based on their stem-like ability to propagate in suspension as spheroids in vitro.

Results: Met\textsuperscript{mt};Trp53fl/+ tumours possessed gene expression profiles that strongly resembled the claudin-low subtype of human TNBC. Interestingly, Met\textsuperscript{mt};Trp53fl/+ tumour cells formed spheres that were only moderately sensitive to pharmacologic Met inhibition. We have identified fibroblast growth factor receptor 1 (FGFR1) signaling as a key compensatory pathway for Met in Met\textsuperscript{mt};Trp53fl/+ TIC, as dual Met-FGFR1 inhibition abrogated sphere formation. In mice transplanted with Met\textsuperscript{mt};Trp53fl/+ cells, combination treatment with Met and FGFR1 inhibitors prolonged tumour-free survival and decreased tumour penetrance and growth rate compared to either inhibitor alone. Consistent with our findings in mouse model, human claudin-low (Basal B) cell lines had the highest co-expression of MET and FGFR1 and were sensitive to dual inhibition of both receptors in sphere-forming assays. We are currently evaluating the efficacy of targeting MET and FGFR1 signaling in candidate patient-derived xenografts in vivo.

Conclusions: Our results support a role for Met and FGFR1 signaling in TIC regulation in the Met\textsuperscript{mt};Trp53fl/+ claudin-low-like tumours. The requirement for Met and FGFR1 signaling for efficient tumour outgrowth highlights the therapeutic potential of inhibiting both receptors in human claudin-low breast cancer.

Outcome/Impact: TIC promote both cancer initiation and relapse. Validating a role for MET and FGFR1 signaling in TIC could provide important insight into TNBC tumourigenesis, as a well as a rationale for combination therapies targeting both MET and FGFR1 in claudin-low breast cancer.
34. SEMAPHORIN 3C IS AN ANDROGEN RECEPTOR-REGULATED GENE

Tam, Kevin J., Kush Dalal, Michael Hsing, Chi Wing Cheng, Yan Ting Chiang, Paul S. Rennie, Martin E. Gleave, and Christopher J. Ong

1Vancouver Prostate Centre, Vancouver, BC; 2Department of Experimental Therapeutics, BC Cancer Agency, Vancouver, BC

Introduction: Prostate cancer (PCa) is the most commonly occurring cancer in North American men, and progression to treatment-refractory stages frequently occurs. The androgen receptor (AR) is a member of the nuclear receptor superfamily of transcription factors, and it is heavily implicated in PCa progression. Transcriptional targets of the AR are not completely described but include genes involved in cell growth and cell fate. The semaphorin family of signaling proteins is a large grouping of cell surface or secreted signaling proteins, which normally function in neurogenesis and embryogenesis. Semaphorins are also implicated in various forms of cancer however mechanisms detailing their involvement are poorly defined. Our work identifies Semaphorin 3C (SEMA3C) as a novel transcriptional target of AR, and establishes a link between SEMA3C and AR-driven PCa disease progression.

Methods: An androgen response element (ARE) in intron 2 of SEMA3C (SEMA3C-ARE) was identified using RSAT DNA analysis software. Androgen responsiveness of SEMA3C was determined using AR-positive LNCaP cells; SEMA3C was measured via quantitative PCR and Western blot analysis. Ectopic expression of AR in LNCaP and PC3 cells was done using AR overexpression plasmids. Electrophoretic mobility shift assays (EMSAs) and chromatin immunoprecipitation (ChIP) were utilized to show recruitment of AR to the SEMA3C-ARE. AR transactivation of the SEMA3C-ARE was shown by luciferase assay.

Results: Our results indicate that androgens and AR overexpression trigger an upregulation of SEMA3C. AR causes a shift of the SEMA3C-ARE oligonucleotide in EMSA, and R1881, a synthetic androgen, triggers the recruitment of AR to the SEMA3C locus. Furthermore, R1881 causes transactivation of the SEMA3C-ARE.

Conclusions: Our results show that SEMA3C is a direct transcriptional target of the AR.

Outcome/Impact: Deregulated AR signaling underpins prostate cancer progression underscoring the need to further elucidate transcriptional targets of AR. Identification of SEMA3C as a novel target of AR provides a potential candidate for targeted therapy.
35. THE PLASMINOGEN RECEPTOR P11 CONTRIBUTES TO KRAS$^{G12D}$-DRIVEN INVASIVENESS OF PANCREATIC CANCER CELLS AND IS UPREGULATED IN HUMAN PANCREATIC TUMOURS

Moamen, Bydoun¹, Weei Huang¹, David M. Waisman¹,²

¹Department of Pathology; ²Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia.

Introduction: Despite its rarity, pancreatic cancer (PC) remains the deadliest cancer type among all cancers with a 5-yr survival rate of only 4%. PC patients are often diagnosed late at which point the cancer has spread to other organs and sabotaged their function leading to patient death.

Rationale: Our laboratory has previously shown that the plasminogen receptor p11 is important for cancers to grow and spread to other organs. In fact, p11 is highly active on invasive cancer cells and acts as a “hub” to activate enzymes which allow cancer cells to chew their way through neighboring fibrotic tissue, invade surrounding tissues, and metastasize. However, the involvement of p11 in PC is not fully understood.

Methods: Here, we investigate the involvement of p11 in PC using three different approaches:
1) Culturing of human PC cell lines which were depleted of p11 (using RNAi technology) followed by in vitro evaluation of p11-depleted cells to generate active enzymes and invade through an artificial matrix using our well-established assays.
2) Utilizing an inducible mouse model that closely mimics the dynamics of PC in human patients.
3) Examining p11 levels in tumours resected from PC patients admitted to the QEII hospital in Halifax.

Results: I have demonstrated that depletion of p11 in human PC cells hampers their ability to activate enzymes and invade by over 60%. Interestingly, p11 expression was found to be driven by oncogenic KRAS, a commonly mutated gene in PC (>95% of cases). We also found that p11 was highly expressed in mouse pancreatic tumours compared to normal pancreata. This increase was also seen in tumours isolated from two patients with invasive PC.

Conclusion: Our data suggests that p11 contributes to the invasive properties of PC cells via enhancing the production of active proteases. This is further supported by the elevated p11 levels seen in human PC samples.

Outcome/Impact: Ultimately, we hope to determine the full contribution of the KRAS/p11 axis to PC metastasis which may position p11 as a “druggable” target and a potential marker to predict outcome in newly diagnosed PC patients.
36. MIR-106A AND MIR-106B AFFECT METASTASIS OF LUNG ADENOCARCINOMA VIA EMT-DEPENDENT AND EMT-INDEPENDENT PATHWAYS

Enfield, Katey S.S.¹, David A Rowbotham¹, Alice Holly², Graham Dellaire², Chiara Pastrello³, Igor Jurisica³, Calum MacAulay¹, Stephen Lam¹, Wan L Lam¹

¹BC Cancer Research Centre, Vancouver BC; ²Dalhousie University, Halifax NS; ³Princess Margaret Cancer Centre, Toronto ON

Introduction: We sought to identify microRNAs (miRNAs) involved in metastatic lung adenocarcinoma (AC). We identified miR-106a and miR-106b, paralogs of the oncogenic miR-17~92, as overexpressed in metastatic lung AC and characterized their ability to regulate metastasis in vitro and in vivo.

Methods: MiRNA expression was deduced from small RNA sequencing data derived from clinical lung AC specimens (60 localized, 27 with lymph node invasion) and paired non-malignant tissues. MiR-106a and miR-106b overexpression vectors and controls were stably transfected into immortalized non-malignant Human Bronchial Epithelial Cells (HBECs) and AC H2347 and H1993 cells by lentiviral delivery. Migration and invasion was assessed by Boyden chamber assay and zebrafish models of metastasis. Cell proliferation was assessed by BrdU incorporation assay. Expression of epithelial-to-mesenchymal transition (EMT) markers and signalling proteins were assessed by Western Blot.

Results: MiR-106a and miR-106b were significantly overexpressed in lung AC with lymph node invasion. Overexpression of miR-106a and miR-106b significantly increased proliferation of H2347 cells. All AC cell lines displayed a marked increase in metastasis in vitro and in vivo, and were associated with increased mesenchymal (H2347) or epithelial (H1993) markers. H2347 cells underwent a morphological change characteristic of EMT.

Conclusions: MiR-106a and miR-106b are overexpressed in metastatic lung AC. Lung AC cell models indicate these miRNAs are metastatic agonists, affecting the metastatic potential of cells through EMT-dependent and EMT-independent mechanisms. The concomitant increase in E-cadherin and metastasis seen in H1993 cells may be indicative of collective invasion, whereby metastatic cells retain cell-to-cell contacts and progress as clusters or sheets rather than single cells.

Outcome/Impact: We have demonstrated miR-106a and miR-106b are important to EMT-dependent and EMT-independent metastasis of lung AC. Deeper characterization of this phenomenon will improve our understanding of metastasis, and may identify novel therapeutic intervention points for lung and other epithelial cancers. With the development of miRNA therapeutics, miR-106a/b may be promising targets to prevent or treat metastatic disease.
37. KSHV MODULATES THE IRE1-XBP1 AXIS OF THE UNFOLDED PROTEIN RESPONSE DURING LYTIC REPLICATION

Johnston, Benjamin P.\textsuperscript{1,2}, Craig McCormick\textsuperscript{1,2}

\textsuperscript{1}Department of Microbiology & Immunology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4R2, \textsuperscript{2}Beatrice Hunter Cancer Research Institute

\textbf{Introduction:} Kaposi’s sarcoma-associated herpesvirus (KSHV) is the infectious cause of the complex endothelial neoplasm Kaposi’s sarcoma, and two rare lymphoproliferative disorders, primary effusion lymphoma (PEL) and multicentric Castleman’s disease. Here we analyzed the role of the cellular stress management program, the unfolded protein response (UPR), during KSHV lytic replication.

\textbf{Methods:} We used multiple KSHV-infected cell lines to monitor IRE1-XBP1 signaling node of the UPR. Lentiviral vectors were employed to determine the effect of XBP1 on lytic replication. Flow cytometry was used to measure viral titres. \textit{xbp1} mRNA splicing was determined by RT-PCR and XBP1s protein levels were measured by western blots. Expression of XBP1s-target genes was determined by qPCR.

\textbf{Results:} We demonstrate that lytic replication in two different KSHV model cell lines, BCBL-1 and iSLK.219 cells, can induce \textit{xbp1} splicing. However, despite \textit{xbp1} splicing, XBP1s protein failed to accumulate in these cells and cellular XBP1s-target genes were not upregulated. Moreover, ectopic expression of XBP1s inhibited the production of infectious viral progeny.

\textbf{Conclusions:} Although XBP1s plays an important role in reactivation from latency, it inhibits later steps in lytic viral replication. Our findings suggest XBP1 may be downregulated by products of the lytic viral gene expression program to mitigate its deleterious effects and permit efficient viral replication.

\textbf{Outcome/Impact:} Identification of a key signalling node in KSHV replication will allow us to develop new therapeutic strategies that may inhibit tumourigenesis.
38. THE IMPACT OF BCL2, BCL6 and MYC TRANSLOCATIONS ON RISK OF TRANSFORMATION IN FOLLICULAR LYMPHOMA

Kridel, Robert¹, Anja Mottok¹, Susana Ben-Neriah¹, Pedro Farinha¹, King Tan¹, Daisuke Ennishi¹, Fong Chun Chan¹, David W. Scott¹, Laurie H. Sehn¹, Joseph M. Connors¹, Marco A. Marra², Sohrab P. Shah³, Christian Steidl¹, Randy D. Gascoyne¹

¹Centre for Lymphoid Cancer; ²Genome Sciences Centre; ³Molecular Oncology, BC Cancer Agency, Vancouver BC

Introduction: In follicular lymphoma (FL), the most common indolent non-Hodgkin lymphoma subtype, the median overall survival time for newly diagnosed patients is currently well beyond 10 years. Nonetheless, in 20-30% of patients, the disease transforms into an aggressive lymphoma subtype, an event associated with increased morbidity and risk of lymphoma-related death. The genetic alterations selected for during the process of transformation are increasingly well understood, but we at present lack robust molecular predictors of transformation.

Methods: We built a tissue microarray (TMA) using the samples from 107 FL patients who experienced transformation within 5 y after the study sample was taken. We also constructed a TMA using the samples from 91 FL patients who experienced neither progression nor transformation for at least 5 y. Translocations in BCL2, BCL6 and MYC were assessed by fluorescence in situ hybridization breakapart assays.

Results: In the cohort of patients who developed transformed lymphoma within 5 y, the prevalence of BCL2 translocations was lower (77% vs 93%, P=0.01) and the prevalence of BCL6 or MYC translocations were higher (21% vs 10%, P=0.07; and 12% vs 1.5%, P=0.02, respectively) than in cases who experienced neither progression nor transformation for at least 5 y. The absence of a BCL2 translocation, the presence of a BCL6 translocation and the presence of a MYC translocation were each significantly associated with a higher cumulative incidence of transformation (P<0.01, P=0.02 and P=0.01, respectively). In a multivariate model, only the absence of a BCL2 translocation was significantly associated with an increased risk of transformation (hazard ratio 2.4, 95% confidence interval 1.3-4.4, P<0.01).

Conclusions: In FL, transformation is more than 2.3 times as likely to occur when BCL2 translocation is absent in the underlying follicular lymphoma than when it is present.

Outcome/Impact: The absence of BCL2 translocations identifies a subset of patients that are at risk of early transformation and therefore candidates for novel, risk-adapted treatment approaches.
39. BIRC6: EMERGING THERAPEUTIC TARGET IN CASTRATION RESISTANT PROSTATE CANCER

Luk, Sze Ue (Iris)¹², Fang Zhang², Hui Xue¹², Hongwei Cheng², Peter W Gout², Martin Gleave¹, Yuzhuo Wang²

¹Vancouver Prostate Centre, Vancouver, BC, Canada; ²Department of Experimental Therapeutics, BC Cancer Agency, Vancouver, BC, Canada

Introduction: Metastatic castration-resistant prostate cancer (CRPC) remains the major clinical challenge in prostate cancer (PCa) and is highly resistant to existing therapies. The Inhibitor of Apoptosis (IAP) family is known to play key roles in promoting cancer cells survival and drug resistance. Our group has reported that BIRC6, a much-less studied IAP member, was elevated in PCa and CRPC, and was important in promoting CRPC survival. Given the pro-survival role, we hypothesize that anti-BIRC6 agent can effectively suppress CRPC growth in vitro and CRPC progression in vivo models, particularly patient-derived xenograft model.

Methods: An Antisense Oligonucleotide (ASO) that specifically knocked down BIRC6 and an additional IAP member was developed. The effects of ASO on proliferation, apoptosis and survival signaling in PCa cells were examined by in vitro functional assays. The therapeutic potential of ASO was studied in vivo using a PC3 xenograft model and a more clinically relevant patient-derived xenograft (PDX) model, LTL313BR. LTL313BR is a castration-resistant tumour line which well recapitulates the histopathological features and phenotypes (e.g. androgen receptor expression) of patient tumour.

Results: Treatment with the anti-BIRC6 ASO resulted in substantial growth inhibition in CRPC cells PC3 and C4-2 and was associated with increased apoptosis, cell-cycle arrest and suppression of NFkB transactivation. Consistently, ASO treatment led to marked suppression of the PC3 tumour growth in the xenograft model. More importantly, we demonstrated that ASO also substantially impeded the growth of the PDX tumour LTL313BR, where LTL313BR was shown to be resistant to bicalutamide and second-generation androgen receptor antagonist enzalutamide.

Conclusions: Using a clinically relevant in vivo model together with in vitro assays, we demonstrated that anti-BIRC6 ASO can suppress CRPC cells survival and effectively hamper progression of CRPC.

Impact: This study provides proof-of-principle data supporting a novel therapeutic agent against CRPC and treatment resistant PCa which may ultimately improve patient survival.

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40. IDENTIFYING TUMOUR SUBPOPULATIONS USING SINGLE-CELL PROFILING OF BREAST CANCER PATIENT-DERIVED XENOGRAFTS

Savage, Paul1,6, Saleh Sadiq M2,6, Iacucci Ernesto5,7, Revil Timothe5,7, Wang Yu-Chang7, Monast Anie6, Bertos Nicholas6, Szulwach Keith8, Chandana Batchu8, Omeroglu Atilla3, Park Morag2,3,4,6, and Ragoussis Jiannis5,7

Departments of 1Experimental Medicine, 2Biochemistry, 3Pathology, 4Oncology, 5Human Genetics, 6Rosalind & Morris Goodman Cancer Research Centre, 7Genome Quebec Innovation Centre, McGill University, Montreal, QC, Canada; 8Fluidigm Corporation, San Francisco, CA, USA

Introduction: Subtyping schemes have been used to extensively describe inter-tumour heterogeneity of breast cancer, yet little is known about the diversity within tumours. Here, we have developed a patient-derived xenograft (PDX) from an index case to identify tumour subpopulations and perform functional assays.

Methods: A PDX was generated by orthotopically engrafting fragments from a freshly resected breast tumour into NOG mice. At endpoint, the PDX was harvested and dissociated to single cells for microfluidic capture, followed by bulk and single-cell genomic profiling. Subtype assignment was performed on RNAseq read counts using absolute intrinsic molecular subtyping. Variant calls were generated from exome sequencing data using GATK and Samtools.

Results: Despite being subtyped as basal-like by bulk gene expression analysis, single-cell RNAseq of 30 cells revealed HER2-enriched and normal-like cells co-existing with the predominant basal-like population. Genes differentially expressed between these cells are involved in proliferation and differentiation. Exome sequencing of 81 single cells revealed clonal mutations in BRCA1 and TP53, in addition to various subclonal mutations. Loss of heterozygosity was observed in 16 genes previously identified by TCGA and putative novel mutations were identified in 7 cancer driver genes.

Conclusions: We have developed a pipeline to model and characterize intra-tumour heterogeneity. Our preliminary data suggests that multiple breast cancer subtypes may co-exist within a given tumour. Ongoing work includes an integrative genomic analysis and functional studies.

Outcome/Impact: The functional significance of intra-tumour heterogeneity is unknown, yet it’s speculated to underlie clinically important phenotypes such as invasion, metastasis and drug-resistance. Here, we have developed a system to interrogate the extent of diversity within human breast tumours and investigate interactions and differential phenotypes between subpopulations, with the possibility of identifying novel therapeutic strategies.
41. IDENTIFYING GENES THAT CO-OPERATE WITH MUTANT P53 & ACTIVATED STAT3 IN BREAST CANCER

Schachter, Nathan F.\(^{1,2}\), Jessica R. Adams\(^2\), Sorana Morrissy\(^2\), Livia Garzia\(^2\), Adam J. Dupuy\(^4\), Michael D. Taylor\(^{2,3}\), Sean E. Egan\(^{1,2}\)

\(^1\)Molecular Genetics, University of Toronto, Toronto, ON, Canada; \(^2\)Developmental & Stem Cell Biology, \(^3\)Arthur and Sonia Labatt Brain Tumour Research Center, SickKids Hospital, Toronto, ON, Canada; \(^4\)Anatomy & Cell Biology, University of Iowa, IA, United States of America.

**Introduction:** Breast cancer (BC) is the leading cause of cancer-related deaths in women worldwide. Tumours with poor prognosis often display two characteristics; mutation of \(TP53\) (which codes for the p53 transcription factor), and constitutive activation of the Stat3 transcription factor. Currently, genetic alterations that co-operate with mutant-p53 or activated-Stat3 in BC remain largely undetermined.

**Methods:** To identify genes that co-operate with mutant-p53 or activated-Stat3, we performed a transposon-based Sleeping Beauty (SB) cancer gene discovery screen in mice. SB screens involve mobilizing transposons engineered to activate, repress, or truncate genes depending on site of insertion and orientation. This system has previously pinpointed genetic alterations associated with leukemia, prostate, colorectal, brain, and hepatocellular cancers and here, is used to study BC. By generating SB mice carrying either Rosa26\(^{LSL-Stat3C}\), mutant \(\text{Trp53}^{LSL-R270H}\), or neither allele, we are able to distinguish genes preferentially altered in the presence of mutant-p53 or active-Stat3. Thus far, over 350 tumours have been collected for analysis. Ligation-mediated PCR combined with next-generation sequencing have identified genes repeatedly targeted for mutagenesis by transposons.

**Results:** 10 genes were identified as being recurrently, clonally altered in the presence of p53 mutations. Of these 10 genes, only one overlapped with the 56 genes frequently disrupted in tumours carrying wild-type p53. Preliminary evidence suggests several mutations found in p53-mutant tumours cause Met receptor over-expression/activation which was confirmed using immunohistochemistry. While genetic analysis of tumours carrying activated-Stat3 is still underway, SB mice carrying this allele exhibit accelerated tumour growth with high penetrance.

**Conclusions:** Preferential alteration of specific genes in a p53-mutant background suggests these mutations co-operate with p53\(^{R270H}\) to drive mammary tumourigenesis. Furthermore, early evidence indicates several of these genetic alterations promote Met signaling. It is also noteworthy that activated-Stat3 significantly enhances BC formation.

**Outcome/Impact:** Upon validating the role of the recurrent alterations identified by this screen, we anticipate identification of novel therapeutic targets for treatment of p53-mutant and activated-Stat3 BC.
42. ASSOCIATION OF RS2282679 A>C POLYMORPHISM IN VITAMIN D BINDING PROTEIN GENE WITH COLORECTAL CANCER RISK AND SURVIVAL

Zhu, Yun¹, Peizhong Peter Wang¹, Guangju Zhai², Roger Green², Sevtap Savas²

Departments of ¹Community Health & Humanities, ²Genetics, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada

Introduction: A single nucleotide polymorphism (SNP) rs2282679 A>C in vitamin D binding protein gene has been associated with lower serum levels of vitamin D. We investigated whether this genetic polymorphism influences colorectal cancer (CRC) risk or mortality and whether the effects vary by vitamin D intake and tumour molecular phenotype.

Methods: A population-based case-control study was performed in 637 CRC incident cases (including 489 follow-up cases) and 489 matched controls in Newfoundland. The cohort was previously genotyped with the Illumina Omni-Quad 1 Million chip in cases and the Affymetrix Axiom® myDesign™ Array in controls. The relationship between the rs2282679 polymorphism and CRC risk was examined using multivariate logistic regressions. For those cases with follow-up data, Kaplan Meier and multivariate Cox models were applied to assess the SNP in relation to CRC overall (OS) and disease-free survival (DFS).

Results: Our data showed no significant association of rs2282679 polymorphism with overall CRC risk. However, we observed some evidence for effect modification of this variant and CRC incidence by total vitamin D intake (P interaction=0.019). Survival analysis showed that the polymorphic C allele of SNP rs2282679 was correlated with poor DFS of CRC (per-allele HR, 1.36; 95% CI, 1.05-1.77). The effect of this SNP on DFS was limited to BRAF wild-type tumours (HR, 1.58; 95% CI, 1.12-2.23). For OS, carriage of the minor C allele conferred an enhanced significant risk for all-cause mortality among patients in higher categories of dietary vitamin D (HR, 2.11; 95% CI, 2.29-3.74; P interaction=0.040), calcium (HR, 1.93; 95% CI, 1.08-3.46; P interaction=0.043), milk (HR, 2.36; 95% CI, 1.26-4.44; P interaction=0.004), and total dairy product intakes (HR, 2.03; 95% CI, 1.11-3.72; P interaction=0.024).

Conclusions: SNP rs2282679 was not associated with susceptibility to overall CRC, but possibly related to decreased DFS after cancer diagnosis. The effect of this SNP on survival among CRC patients varied by vitamin D, calcium, and tumour BRAF mutation status.

Outcome/Impact: The genotype at the CG rs2282679 locus, along with vitamin D and BRAF mutation status, has potential utility as a susceptibility and prognostic biomarker of CRC.
43. **NUCLEAR FACTOR I/B AS A CRITICAL REGULATOR OF LUNG DEVELOPMENT AND LUNG ADENOCARCINOMA PATHOGENESIS**


1BC Cancer Research Centre, Vancouver BC; 2Princess Margaret Cancer Center, Toronto ON; 3University of British Columbia, Vancouver BC

**Introduction**: Genes involved in fetal lung development are thought to play crucial roles in the malignant transformation of adult lung cells. Consequently, the study of lung tumour biology in the context of lung development has the potential to reveal key regulatory pathways reactivated in lung cancer.

**Methods**: 131 pairs of non-small cell lung cancer (NSCLC) tumour and non-malignant lung tissues and 15 human fetal lung tissue samples were profiled by miRNA-sequencing. To investigate protein-coding genes controlled by the *oncofetal* miRNAs identified (miRNAs highly expressed in fetal and tumour tissues), miRDIP algorithm was applied followed by luciferase-reporter assays. Associations between patient survival and mRNA expression of selected *oncofetal* miRNA-gene targets were evaluated in ~1,400 NSCLC cases. Immunohistochemical analysis of *oncofetal* miRNA targets was performed on a lung adenocarcinoma (LUAD) tissue microarray.

**Results**: We describe for the first time a comprehensive characterization of miRNA expression in human fetal lung tissue, and identified numerous miRNAs that recapitulate their fetal expression patterns in NSCLC. Assessment of genes regulated by these *oncofetal* miRNAs led us to identify Nuclear Factor I/B (NFIB), a transcription factor essential for lung development, as being frequently targeted by *oncofetal* miRNAs. Concordantly, analysis of NFIB mRNA expression in multiple NSCLC cohorts revealed its frequent underexpression in tumours (>60%). Remarkably, low expression of NFIB was significantly associated with poorer survival in LUAD patients, consistent with the functional role of NFIB in distal lung cell maturation (i.e. cells that are the precursors of LUAD). Finally, analysis of NFIB protein expression in LUAD specimens revealed that tumours presenting lower levels of this gene are associated with higher grade, biologically more aggressive subtypes of LUAD.

**Conclusions**: This work has revealed a prominent mechanism for the downregulation of NFIB, a developmental transcription factor essential for lung differentiation, which we found to be associated with aggressive phenotypes of LUAD and consequently, poor patient survival.

**Outcome/Impact**: Restoration of NFIB expression in LUAD may induce lung cell differentiation, and therefore has the potential to reduce tumour aggressiveness.
44. DELETION OF TUMOUR SUPPRESSORS Rb AND p53 VIA WAP-CRE INDUCES BRAIN TUMOURS WITH FEATURES OF GROUP 3 MEDULLOBLASTOMA

Chung, Philip E.D.1,2, Remke Marc1,3, Ghanbari Ronak1,3, Gendoo Deena3, Dubuc Adrian1,3, Tsui Jenniffer2, Jiang Zhe2, Liu Jeff3, Shih David1,3, Garzia Livia3, Croul Sidney1,3, Taylor Michael D.1,3, Zacksenhaus Eldad1,2

1Dept. of laboratory medicine and pathobiology, University of Toronto; 2Division of advanced diagnostic, Toronto General Hospital; 3Arthur and Sonia Labatt Brain Tumour Research Center, The Hospital for Sick Children; 4Dept. of Neuropathology, University Health Network

Introduction: Group 3 medulloblastoma (MB) is a highly aggressive pediatric brain cancer. Preclinical mouse models for this tumour may help uncover new therapeutics.

Method: We used cre-loxP system to delete Rb and p53 (or express p53-R270H mutation) via a whey acidic protein (WAP)-Cre transgene. Histology, immunohistochemistry (IHC) and bioinformatics analyses were performed to classify these tumours. Metastasis to the spinal cord and brain was detected by histology and MRI, and confirmed by marker or reporter analysis, using mT/mG transgenic mice. Various stages were analyzed to identify the cell of origin. Three tumour lines were established and different drugs were tested in vitro to identify therapeutic targets.

Results: Rb/p53-deleted (100%, n=124) and Rb-deleted/p53-mutated (100%, n=43) mice developed brain tumours with features of MB, and with a median latency of 130 days and 135 days, respectively. Microarray analysis showed that Rb/p53-deleted tumours most closely resemble Group 3 MB. Rb-deleted/p53-mutated tumours (72.7%, n=11) disseminate to the spinal cord and/or the brain more often than Rb/p53-deleted tumours (15.4%, n=26). Preliminary data suggest the tumours originate from midbrain/cerebral cortex and then migrate to the cerebellum. Of several candidate therapeutics, gemcitabine had the greatest inhibitory effect in vitro. In vivo analysis of gemcitabine is ongoing using our pre-clinical models.

Conclusions: We have generated a mouse model that spontaneously forms tumours with features of Group 3 MB. Our results suggest that these tumours, like WNT MB, which originate in the brain stem, are initiated outside of the cerebellum.

Outcome/Impact: Targeted therapy for Group 3 MB patients would greatly reduce side effects and improve overall survival. This mouse model can be used to study the cell of origin of these tumours and identify new drugs for this devastating disease.
45. MIR-17-92 REPRESSION OF LKB1 IS NECESSARY FOR MYC-DRIVEN METABOLIC REPROGRAMING IN LYMPHOMA

Izreig, Said\textsuperscript{1,2}, Bozena Samborska\textsuperscript{1,2}, Carine Lussier\textsuperscript{1,3}, Thomas F. Duchaine\textsuperscript{1,3}, Russell G. Jones\textsuperscript{1,2}

\textsuperscript{1}Goodman Cancer Research Center, McGill University; \textsuperscript{2}Department of Physiology, McGill University; \textsuperscript{3}Department of Biochemistry, McGill University

Introduction: The proto-oncogenic transcription factor Myc is commonly implicated in human cancers, and is able to orchestrate a pro-cancer metabolic shift when deregulated. While Myc itself has proven to be difficult to target directly, downstream effectors of Myc engaged in metabolic reprogramming in cancer may be more amenable to therapy. The miRNA cluster miR-17-92 is a transcriptional target of Myc, and is potentially involved in metabolic regulation in cancer.

Methods: To investigate the molecular basis of Myc-driven metabolic reprogramming, a Myc-driven lymphoma model with conditional deletion of miR-17-92 was employed. Mass isotopomer labelling coupled to gas chromatography – mass spectrometry was employed to investigate flux of labelled metabolites through multiple metabolite pools. The Seahorse Extracellular Flux Analyzer and NOVA bioprofiler were also employed for real-time measurements of cellular metabolic activity.

Results: Deletion of miR-17-92 caused significant decreases in glycolytic and oxidative metabolism. The miR-17 component of the cluster was found to negatively regulate the master metabolic regulator and tumour suppressor LKB1. Knocking down expression of LKB1 in cells lacking miR-17-92 rescued metabolic activity and tumourigenicity in vivo. Reduced LKB1 expression has been shown to sensitize cells to phenformin, a drug used previously for diabetes. In vivo administration of phenformin to tumour bearing mice dramatically reduced morbidity, and in some cases cured mice of lymphoma.

Conclusions: The powerful metabolic reprogramming engendered by Myc is due, in part, to miR-17-92. Therapeutic intervention using phenformin is rationalized and demonstrated to be effective as a single agent in lymphoma bearing mice.

Outcome/Impact: By investigating downstream effectors of Myc activity, miR-17-92 and LKB1 signaling have been identified as being essential in Myc-driven metabolism in lymphoma. Given this understanding, metabolic therapies aimed at these molecular targets may provide new avenues for treatment of Myc-driven lymphomas.

This work was supported by grants from the Terry Fox Research Foundation (TFF-116128) and the CIHR (MOP-93799) (to R.G.J).
46. RAS/MEK DOWNREGULATES IRF1 EXPRESSION TO BLOCK THE IFN INDUCED ANTI-VIRAL RESPONSE

Komatsu, Yumiko ¹, Sherri Christian², Nhu Ho¹, Theerawat Pongnopparat¹, Maria Licursi¹, and Kensuke Hirasawa¹

¹Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada; ²Department of Biochemistry, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada

Introduction: Oncolytic viruses exploit common molecular changes in cancer cells, which are not present in normal cells, to target and kill cancer cells. Ras transformation and defects in type I interferon (IFN)-mediated antiviral responses are known to be the major mechanisms underlying viral oncolysis. Previously, we demonstrated that oncogenic RAS/Mitogen-activated protein kinase kinase (Ras/MEK) activation suppresses the transcription of many IFN-inducible genes (MEK-downregulated IFN-inducible (MDII) genes) in human cancer cells, suggesting that Ras transformation underlies type I IFN defects in cancer cells. Here, we investigated how Ras/MEK downregulates IFN-induced transcription.

Methods: Promoter deletion analysis was conducted to identify promoter regions responsible for transcriptional regulation of the MDII genes by Ras/MEK. Mouse embryonic fibroblasts (MEFs) were established from wild type mice and IRF1⁻/⁻ mice to determine IRF1 involvement in the regulation of the MDII genes. MEK inhibitors and RNAi oligos were used to determine whether Ras/MEK regulates IRF1 expression. To determine whether IRF1 plays roles in viral oncolysis, human cancer cells transfected with pCMV-SPORT6 control or pCMV-SPORT6-IRF1 were infected with the oncolytic vesicular stomatitis virus (VSV-M51R).

Results: IRF1 binding site was identified as the promoter region responsible for the regulation of transcription by MEK. MEK inhibition promoted transcription of the MDII genes in wild type MEFs, but not in IRF1⁻/⁻ MEFs. Furthermore, IRF1 protein expression was lower in RasV12 transformed NIH3T3 cells compared with vector control NIH3T3 cells, but was restored to equivalent levels by inhibition of MEK. Similarly, the restoration of IRF1 expression by MEK inhibition was observed in human cancer cells. IRF1 re-expression rendered cancer cells resistant to infection by VSV-M51R.

Conclusions: Together, this work demonstrates that Ras/MEK activation in cancer cells downregulates transcription of IFN-inducible genes by targeting IRF1 expression, resulting in increased susceptibility to viral oncolysis.

Outcome/Impact: Although IRF1 is a well-known tumour suppressor and antiviral protein, its involvement in viral oncolysis has not been investigated. Our findings will contribute toward the design of novel oncolytic viruses and their therapeutic uses for cancer treatment with improved efficacy and safety.
47. INTEGRATIVE TRANSCRIPTOME SEQUENCE ANALYSIS OF TREATMENT RESISTANT PEDIATRIC ACUTE MYELOID LEUKEMIA

Lim, Emilia\(^1\), Yussanne Ma\(^1\), Erin Pleasance\(^1\), Heather Schuback\(^2\), Daniela Gerhard\(^4\), Robert Arceci\(^3\), Soheil Meshinchi\(^2\), Marco Marra\(^1\)

\(^1\)Genome Sciences Centre, Vancouver BC; \(^2\)Fred Hutchinson Cancer Research Center, Seattle WA; \(^3\)Ron Matricaria Institute of Molecular Medicine, Phoenix AZ; \(^4\)Office of Cancer Genomics, National Cancer Institute, Bethesda MD

**Introduction:** Induction chemotherapy results in complete remission in 80-90% of children with acute myeloid leukemia (AML). However, a subset of patients whose leukemia is refractory to induction therapy or suffer from relapse have a dismal outcome.

**Method:** As part of a comprehensive genome-scale approach to identify prognostic markers and therapeutic targets, we performed mRNA-seq and miRNA-seq analyses for 278 primary (taken at diagnosis), 28 refractory and 47 relapse samples. To screen for miRNA:mRNA interactions that could be functional in pediatric AML, we identified miRNA:mRNA pairs with anti-correlated expression profiles and miRNA binding site predictions consistent with miRNA:mRNA interaction.

**Results:** Non-negative matrix factorization (NMF) of mRNA expression profiles revealed 5 subgroups of patients, where 1 subgroup is further distinguished by abundant expression of ribosomal proteins and superior survival. Analysis of the mRNA data revealed that Ribosomal Protein L28 is less abundant in relapse samples than in primary samples (Wilcox test q-value <0.05), suggesting a dysregulation of protein translation at relapse. Analysis of the miRNA data revealed that abundant miR-106a-363 expression is associated with inferior overall survival (Cox PH q-value <0.05), suggesting that is associated with treatment resistance. In agreement with this, miR-106a-363 is more abundant in relapse and refractory samples than in primary samples (Wilcox test q-value <0.05). In addition, integrative miRNA:mRNA expression analyses further revealed that targets of miR-106a-363 are involved in oxidative phosphorylation, a process that is suppressed in treatment-resistant leukemic cells\(^1\).

**Conclusions:** Our comprehensive sequence analysis of the pediatric AML transcriptome identifies characteristic mRNA/miRNA expression profiles that are associated with treatment resistance, and reveals regulatory networks of significance for leukemogenesis.

**Outcome/Impact:** Prognostic mRNAs/miRNAs are suitable candidates for diagnostic and preemptive intervention assays for pediatric AML patients, while dysregulated mRNA/miRNAs and regulatory networks are potential therapeutic targets of pediatric AML treatment.

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48. DOWNREGULATION OF CD24 EXPRESSION BY ONCOGENIC RAS

Pallegar, Nikitha K., D. Craig Ayre and Sherri L. Christian

Department of Biochemistry, Memorial University of Newfoundland, St. John’s, NL, Canada

Introduction: Breast cancer is one of the most common cancers among Canadian women, with 1 in 4 women diagnosed per year. Tumours have many different cell types that contribute to cancer formation and progression. Breast cancer stem cells (BCSCs) within the tumour can initiate tumour formation, have high invasive properties, favor metastasis, and often express the Ras oncogene. BCSCs can be identified by their high expression of CD44 and ALDH with low expression of CD24 (CD44+/CD24-/ALDH+). CD24 expression is dynamically regulated in breast cancer cells with CD44+/CD24+ cells readily giving rise to CD44+/CD24- cells and vice versa. Therefore, alterations in CD24 expression can alter disease progression. However, the mechanism that regulates CD24 expression is not known.

Methods: We analyzed the CD24 mRNA and protein expression levels in RasV12 cells, where oncogenic Ras is constitutively activated. We analyzed the contribution of signal transduction pathways downstream of Ras on CD24 expression using RasV12 effector mutants. Next, we then inhibited the Raf and PI3K pathways by treating RasV12 cells with inhibitors, to determine if inhibition of these pathways could restore CD24 expression. Using luciferase reporter assays, we assessed the regulation of the CD24 promoter region by oncogenic Ras. Finally we analysed the relationship between expression levels of CD24 and Ras-related in various breast cancer cells using published transcriptomics data.

Results: We found that CD24 mRNA and protein expression was decreased by oncogenic Ras mediated by a 688bp region of CD24 promoter in a model system. Activation of either the PI3K or Raf pathway, downstream of Ras, was sufficient to repress CD24 expression. However, inhibition of either of these pathways was not sufficient to fully restore CD24 expression. In support of this observation, we found that CD24 is inversely correlated to Ras pathway genes in triple negative breast cancer cells.

Conclusion: Therefore, oncogenic Ras efficiently represses CD24 expression via mechanisms that do not rely on continual activation of downstream signal transduction pathways.

Outcome/Impact: Ras signaling regulates CD24 surface expression, which is an important regulator of BCSC function. By elucidating the mechanism used by Ras to regulate CD24 expression, the population of the breast tumour that are BCSCs could potentially be regulated.
49. SNAPSHOT OF ACTIVE SURVEILLANCE TRENDS FOR PROSTATE CANCER: A CROSS-SECTIONAL STUDY RESULTS BY PROVINCE IN CANADA

Timilshina, Narhari1, Shabbir M.H. Alibhai1, Veronique Ouellet2, Nathalie Delvoye2, Darrel Drachenberg2, Antonio Finelli1, Marie-Paule Jamma12, Hélène Lapointe2, Kenny Lynch2, Anne-Marie Mes-Masson2, Jean-Benoît Paradis2, Paula Sitarik2, Alan So2, Fred Saad2

1University Health Network, 2The Canadian Prostate Cancer Biomarker Network

Introduction: In active surveillance (AS), practitioners delay curative treatment strategies in low-risk patients until there is evidence of disease progression, at which time active treatment is initiated. Although the uptake of AS appears to be increasing, the actual uptake in Canada remains largely unknown.

Methods: We used database interrogation approach in four different provinces (BC, QC, MB and ON) to evaluate the use of AS in men who underwent a biopsy in 2010. Following diagnosis, patients were divided into incident and prevalent cases. Clinical and pathological information were collected for 12 months following the last biopsy of 2010. Eligibility for AS and treatment trends by region were compared using chi-square test.

Results: Of 941 patients, 645 patients were eligible for AS with median Charlson score of 0, of whom over two-thirds (68.1%) chose AS at diagnosis. Patients undergoing AS varied in mean age by region from 61.8 to 65.4 years (p=0.007). There were significantly differences in use of AS by region (BC 63.1%, MB 100%, Montreal QC 72.9%, Chicoutimi QC 43.3%, Laval QC 57.5%, p=0.001).

105 (16.3%) were re-biopsied within 1-year and rates ranged considerably from 5.0-29.8%. Among those who underwent re-biopsy, 26.4% of patients showed increased disease risk (17.6% Gleason reclassification, 20.9% volume reclassification) and this change in risk varied by region (ranging from 0-44.4%).

Conclusions: These results suggest that AS is commonly practiced in large parts of Canada, although there are significant differences in practice patterns between and within provinces. More in-depth analyses will be required to understand the root causes of these differences, and also to determine whether AS uptake is changing over-time.

Outcome/Impact: The study result shows significant uptake of this management approach but important differences in patterns of AS use between provinces. The larger study will focus on the identification of markers and other factors that predict risk in order to inform clinical management decisions, in particularly AS. In addition, our study provides important baseline data on Canadian rates of AS use in low-risk prostate cancer.
50. SEMAPHORIN 3C-INDUCED INCREASE IN STEROIDOGENESIS IS IMPLICATED IN THE PROGRESSION OF CRPC

Yenki, Parvin1,2, Chi Wing Cheng1, Hans Adomat1, Martin Gleave1, Christopher Ong1,2

1Vancouver Prostate Centre, Vancouver, BC; 2Department of Surgery, University of British Columbia, Vancouver, BC.

Introduction: This project aims to study the biological role of Semaphorin3C (SEMA3C) in castration resistant prostate cancer (CRPC) progression. We have found that SEMA3C is a key driver of prostate cancer (PCa) growth through activation of EGFR and cMet receptor tyrosine kinase (RTK) pathways. RTK signalling activation has been shown to induce de novo steroidogenesis, therefore we hypothesize that SEMA3C-mediated RTK signalling may contribute to CRPC progression through promotion of androgen biosynthesis in androgen-deprived PCa cells.

Methods: LNCaP and 22Rv1 cells stably overexpressing SEMA3C were compared to mock transfected cells with respect to their in vitro steroidogenesis and also steroidogenic enzyme expression levels; hormone were measured by mass spectrometry and steroidogenic enzyme expression by quantitative PCR. De novo steroidogenesis was detected by pulsing cells with C14-labeled acetate as a steroid precursor; production of intermediates was measured by scintillation. To measure intratumoural androgen level, athymic nude mice will be subcutaneously xenografted with LNCaP-overexpressing and mock transfected cells followed by castration. Tumour homogenates’ steroids will then be extracted and run on LC/MS system.

Results: Steroid analysis showed a significant increase in secreted testosterone and dihydrotestosterone in conditioned media of SEMA3C-overexpressing cells. SEMA3C overexpression also upregulated expression of steroidogenic enzymes CYP17A1, AKR1C3 and SRD5A1. Steroid analysis of SEMA3C-overexpressing LNCaP cells incubated with C14-labeled acetate showed elevated levels of the de novo steroidogenic intermediate androsterone.

Conclusions: These findings indicate that SEMA3C contributes to an adaptive response to androgen deprivation, namely steroidogenesis, thereby maintaining AR signaling in CRPC cells.

Outcome/Impact: Androgen-deprivation therapy is not curative in CRPC stage, thus creating a major therapeutic challenge in PCa treatment. Understanding the mechanism of SEMA3C function as a key molecule could be useful in the design of novel treatment strategies. We aim to investigate SEMA3C cross-talk with other steroidogenic signaling pathways and its potential role in epithelial-stromal cell interaction.
51. CHARACTERIZATION OF GENOMIC SOMATIC ALTERATIONS IN TRANSFORMED AND TREATMENT RESISTANT FOLLICULAR LYMPHOMA

Chan, F.C.1,2, Kridel R.1, Mottok A.1, Boyle M.1, Farinha P.1, Meissner B.1, Shumansky K.5, Aniba R.5, Scott DW1, Sehn LH.1, Connors JM.1, Steidl C1, Gascoyne RD1, Marra M1,3,4, Shah S1,5

1Centre for Lymphoid Cancer, BCCA; 2Bioinformatics Graduate Program, UBC; 3Genome Sciences Center, BCCA; 4Department of Medical Genetics, UBC; 5Department of Molecular Oncology, BCCA

Introduction: Follicular lymphoma (FL) is the most common form of indolent lymphoma. Despite increasing knowledge of the somatic genomic alterations associated with FL, these findings do not explain: 1) the transformation of FL to aggressive disease, and 2) treatment resistance. In this study we seek to identify the somatic genomic alterations associated with transformation and treatment resistance in FL.

Method: We assembled a study cohort consisting of 41 patients separated into 3 different categories: 15 transformed FL (TFL), 6 progressed FL (PFL), and 20 non-progressed FL (NPFL). For TFL and PFL patients, we obtained primary biopsies (T1; taken at diagnosis), biopsies at transformation/progression (T2), and matched normal samples. For NPFL patients, we obtained tumour (T1) and matching normal samples. For each biopsy, we performed whole genome sequencing generating a total of 103 libraries.

Results: Across all biopsies (n = 62), the most recurrently mutated genes were CREBBP (71%), MLL2 (55%), and EZH2 (30.6%) whereas the most recurrently rearranged genes were BCL2 (79%), FHIT (25%), and BCL6 (13%). The mean mutational load of T2 biopsies (8275 ± 2278) was significantly higher than T1 biopsies (6867 ± 3586; P < 0.001). Likewise, the mean structural rearrangement load in T2 biopsies (42.8 ± 31.3) was significantly higher than T1 biopsies (26.5 ± 22.1) (P < 0.003). Analysis of mutations and rearrangements in only T1 biopsies revealed mutations in HIST1H1E (12.2%), UNCP80 (7.3%), and FAS (7.3%) as well as rearrangements in GRM1 (12%) and SGK1 (5.5%) to be exclusive to TFL and PFL patients but not found in NPFL patients.

Conclusion(s): Our study describes genetic alterations in primary and transformed/progressed FL. Additionally, we identified recurrent coding mutations and rearrangements found exclusively in TFL and PFL patients suggesting such alterations may contribute to transformation and treatment resistance of FL.

Outcome/Impact: This comprehensive sequencing analysis will lead to the development of molecular predictors of poor outcome and potentially novel targets for therapy.
52. HIGH-CONTRAST PHOTOACOUSTIC IMAGING OF PROSTATE CANCER USING A BIOMIMETIC PORPHYRIN NANOPARTICLE

Charron, Danielle M.¹,², Chen Juan¹, Zheng Gang¹,²

¹Princess Margaret Cancer Centre & Techna Institute, University Health Network, Toronto; ²Institute of Biomaterials and Biomedical Engineering, University of Toronto

Introduction: A major paradigm shift in prostate cancer (PC) treatment is emerging to deliver safe and effective localized treatment of the dominant tumour foci. Under this new paradigm, there is a need for high-contrast, tumour-targeted imaging agents to improve identification and delineation of the target lesion.

Methods: We have developed a biomimetic nanoparticle contrast agent for photoacoustic (PA) imaging of PC and assessed its performance in vivo using a PC-3 orthotopic model. Porphyrin-lipid derived from natural algae chlorophyll is incorporated by self-assembly into a high-density lipoprotein-like nanoparticle. Porphyrin nanoparticle biodistribution and tumour selectivity is assessed by porphyrin fluorescence of ex vivo tissues. The optimal time window for tumour delineation is identified by monitoring the porphyrin nanoparticle PA signal within the tumour region following i.v. injection.

Results: Porphyrin-lipid supramolecular arrangement enabled by the compact lipid and protein scaffold generates an intense absorption band red-shifted from 750 nm to 824 nm, with PA activity at both wavelengths. Prostate tumour was delineated in vivo with significantly greater PA contrast enhancement at 824 nm than at 750 nm due to the deeper light penetration and lack of interference with blood.

Conclusions: PC was successfully delineated in vivo by PA imaging using a novel porphyrin nanoparticle exhibiting wavelength-dependent high-contrast signal intrinsic to the nanoparticle structure. Porphyrin nanoparticle provides a means to improve the effectiveness of PA imaging of PC to accurately delineate the target lesion for focal therapies.

Outcome/Impact: Focal therapy promises to provide a favorable balance between quality-of-life and the risk of disease progression. Medical imaging plays a large role in identifying the target lesion and guiding therapies; however, with current imaging modalities there is a risk of inadequate delineation resulting in residual tumour following treatment. PA imaging using porphyrin nanoparticle provides a means to reduce the risk of developing incurable disease.
53. DNA REPAIR GENES AS POTENTIAL BIOMARKERS OF PARP INHIBITORS IN OVARIAN CANCER

Fleury, H.1*, Carmona E.1, Masson JY.2, Tonin P.3, Provencher, D.1, Mes Masson A-M.1

1CRCHUM/Institut du cancer de Montréal; 2Centre de recherche en cancérologie de l’Université Laval, Quebec; 3The Research Institute of the McGill University Health Centre

Introduction: Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer in North America largely due its late detection (80% with stade III) and high recurrence rate (~70%). In order to improve EOC survival and decrease chemoresistance, several adjuvant drugs are now in clinical trials. In a phase II clinical trial, Poly-ADP Ribose Polymerase inhibitor (PARPi) demonstrated a good efficacy in high grade serous (HGS) EOC patients as expected in patients harboring BRCA1/2 mutations but also was effective in a subset of patients with WT BRCA genes. We hypothesized that other DNA repair pathways maybe important in this response and could potentially serve as predictive biomarkers.

Methods: PARPi sensitivity, in novel HGS-EOC cell lines derived by our group, was determined by clonogenic assay. An integrated analysis with the use of microarray data was performed to identify molecular pathways and genes clustering based on carboplatin or PARPi sensitivity. DNA repair pathways were evaluated by functional assays. Validation of target genes was performed in cell lines using a siRNA approach.

Results: Our preliminary results identified three groups of Olaparib sensitivity in HGS-EOC cell lines; i.e. highly sensitive (0.0001-0.2 µM), sensitive (0.3-4 µM), and resistant (5-28 µM). The overall pathway analysis of the microarray data indicate that the major DNA repair pathways, NHEJ, MMR, HR and NER are differentially expressed in highly sensitive and resistant cell lines. We have focused on three genes in particular, two (MLH1/3) specific to MMR and one ERCC8 implicated in NER. We observed that MLH1 or MLH3 and ERCC8 siRNA silencing in HGS-EOC cell line induced an approximate 10-fold decrease in the Olaparib IC 50 for resistant cell line.

Conclusions: Our results demonstrate that PARPi response is influenced by multiple DNA repair pathways and suggest that these genes need to be included in a more rational selection of patient who may responds to PARPi.

Outcome/Impact: In addition to understanding the bases for PARPi mode of action, this research will contribute to the identification of specific predicting biomarker that can be used for patient selection.
**54. DISSECTING THE ROLE OF NATURAL KILLER T CELL DERIVED IFN-γ IN TUMOUR CONTROL**

**Gebremeskel, Simon**¹ ⁴ Drew Slauenwhite¹, Brent Johnston¹ ² ³ ⁴

¹Department of Microbiology & Immunology, ²Department of Pediatrics, ³Department of Pathology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 1X5; ⁴Beatrice Hunter Cancer Research Institute, Halifax, Nova Scotia, Canada, B3H 4R2

**Introduction:** Natural killer T (NKT) cells are glycolipid-reactive T cells that play important roles in immunosurveillance and immune regulation. However, mechanistic studies examining the role of NKT cells in cancer control are hindered by a lack of tools to target molecules specifically in NKT cells.

**Methods:** To address these issues, we developed a novel protocol to expand functional NKT cells and stably reconstitute them in NKT cell deficient Jα18⁻/⁻ mice. Adoptive transfer of α-galactosylceramide (α-GalCer)-loaded dendritic cells expanded NKT cells in wild-type mice without skewing CD4 or TCR Vβ expression profiles.

**Results:** Expanded NKT cells exhibited enhanced IFN-γ and IL-4 responses upon re-stimulation with glycolipid or CD3 ligation. Adoptive transfer of recently expanded wild-type or IFN-γ⁻/⁻ NKT cells protected recipient Jα18⁻/⁻ mice from B16 melanoma metastasis without the need for additional glycolipid stimulation. However, NKT cell reconstitution in recipient Jα18⁻/⁻ mice was short lived. Long term reconstitution was only achieved when expanded NKT cells were transferred into sublethally irradiated recipients. Thirty days after transfer, NKT cell activation phenotype and α-GalCer-induced cytokine responses were equivalent to naive wild-type mice. Jα18⁻/⁻ recipients reconstituted with wild-type or IFN-γ⁻/⁻ NKT cells were both protected from B16 melanoma metastasis by α-GalCer treatment, and NK cell transactivation was intact in mice reconstituted with IFN-γ⁻/⁻ NKT cells.

**Conclusions:** These studies validate the use of reconstitution protocols to investigate mechanisms of NKT cell immune function, demonstrating that NKT cell-derived IFN-γ and the altered TCR repertoire in Jα18⁻/⁻ mice do not impact NKT cell-mediated anti-tumour responses.

**Outcome/Impact:** Clinically, IFN-γ is used as a prognostic factor in NKT cell based therapy. Our studies suggest that NKT cell derived IFN-γ is not required for protection against tumours. This would not be possible without the new NKT cell reconstitution protocol we have developed.
55. CATALYTIC POCKET INACCESSIBILITY OF ACTIVATION INDUCED CYTIDINE DEAMINASE (AID) IS A SAFEGUARD AGAINST EXCESSIVE MUTAGENIC ACTIVITY

King, Justin J., Courtney A. Manuel, Crystal V. Barrett, Susanne Raber, Heather Lucas, Patricia Sutter and Mani Larijani

Immunology and Infectious Diseases Program, Division of Biomedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, Canada

Introduction: Activation-induced cytidine deaminase (AID) mutates cytidine to uridine at immunoglobulin loci to initiate secondary antibody diversification but also causes genome-wide damage involving many oncogenes. We previously demonstrated that AID has an unusually low catalytic rate when compared to most enzymes. The structure of AID has not been solved.

Methods: Molecular modelling (I-TASSER) and docking (Swiss-Dock) were used to predict the structure of AID in complex with DNA. Using these AID-DNA complexes as a guide, we constructed a library of over 300 AID mutants and chimeras. Our alkaline cleavage assay was used to compare the enzymatic activity of AID variants.

Results: Docking revealed that the majority of AID:DNA complexes would be inactive due to substrate binding such that a cytidine is not positioned for deamination. Furthermore, we found that most AID conformations exhibit fully or partially occluded catalytic pockets. We constructed mutant and chimeric AID variants predicted to have altered catalytic pocket accessibility dynamics and observed significant correlation with catalytic rate.

Conclusions: Here we provide a comprehensive and dynamic model of AID across multiple conformations in complex with DNA. Data from modelling simulations and functional tests of AID variants support the notion that catalytic pocket accessibility is an inherent bottleneck for AID activity.

Outcome/Impact: Due to its role in lymphomagenesis and exacerbation of cancer phenotypes, AID is a potential candidate for anti-cancer therapeutics. Here we describe the molecular surfaces of AID that can be targeted by small-molecule inhibitors. Our finding that the majority of AID conformations are “catalytically restricted” suggests that effective inhibitors which lock AID into these inactive states may be more effective drugs.

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56. INVESTIGATING THE ROLE OF SERINE FOR T CELL ACTIVATION AND MALIGNANCY

Ma, Eric$^{1,2}$ and Jones, Russell G.$^{1,2}$

$^1$Goodman Cancer Research Centre; $^2$Department of Physiology, McGill University, Montreal

Introduction: Metabolism of serine supports cancer progression due to its incorporation into multiple biosynthetic pathways. Activated T cells undergo metabolic changes similar to those displayed in cancer cells, leading to our hypothesis that serine metabolism is required for T cell proliferation, function, and malignant transformation. A comparison of serine metabolism between activated and cancerous T cells will clarify the role of this essential metabolite in health and in disease.

Methods: Using flow cytometry, we can investigate the role of exogenous serine on T cell proliferation, viability, and cytokine production. Additionally, using isotopomer labelling paired with gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry, we are able to observe the contribution of serine to various intracellular metabolite pools.

Results: We observe a significant decrease in proliferation of T cells in the absence of serine. The addition of formate, a metabolite that feeds into the one-carbon metabolism pool, is sufficient to rescue the defect in proliferation. Interestingly, T cells cultured in the absence of exogenous serine do not display a decrease in cytokine production.

Conclusions: In activated T cells, serine metabolism plays an important role in proliferation by fuelling the one-carbon pool for nucleotide biosynthesis. The role of one-carbon metabolism in malignant T cells warrants study, and may prove to be a metabolic sensitivity that is amenable to treatment.

Outcome/Impact: Serine metabolism is actively engaged in some cancer cells, offering the cells a significant growth advantage. From our initial work, it appears serine metabolism is also engaged in activated T cells. An examination of serine’s contribution to one-carbon metabolism in malignant T cells will justify a rational therapeutic intervention based on metabolic sensitivity.

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57. THE COMBINATION OF DOCETAXEL AND ANEUSTAT SYNERGISTICALLY INHIBITS PROSTATE CANCER GROWTH AND METASTASIS - LEADING TO BETTER PROGNOSIS OF PATIENTS

Qu, Sifeng1,2,3, Yuzhuo Wang1,2,3,4

1Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver; 2Vancouver Prostate Centre, Vancouver; 3Interdisciplinary Oncology Program, Faculty of Medicine, University of British Columbia, Vancouver; 4Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver

Introduction: The current first-line treatment for metastatic prostate cancer, the incurable stage for patients, i.e. docetaxel-based therapy, is only marginally effective. The aim of the present study was to determine whether the combination of docetaxel and Aneustat, with synergistic effect in a patient-derived, advanced prostate cancer tissue xenograft model, could inhibit prostate cancer growth and metastasis.

Methods: Human metastatic, androgen-independent C4-2 prostate cancer cells and NOD-SCID mice bearing PTEN-deficient, metastatic and PSA-secreting, patient-derived subrenal capsule LTL-313H prostate cancer tissue xenografts were treated with docetaxel and Aneustat, alone and in combination. Culture growth and xenograft size were determined and animal health monitored. Wound-healing assay in C4-2 and local invasion detection in 313H were performed for metastasis inhibition analysis. Xenografts were profiled using gene expression microarrays. Molecular functions were performed on differentially expressed genes (≥ 2-fold difference) using Ingenuity Pathway Analysis software (IPA). Clinical outcome and metastatic prognosis association in prostate cancer patients were confirmed in Oncomine database.

Results: In vitro, the combination of docetaxel and Aneustat synergistically inhibit C4-2 cell replication in a dose-dependent manner (combination index < 1). In vivo, with consistency, the combination of docetaxel and Aneustat enhanced anti-tumour activity synergistically and markedly, without inducing major host toxicity. Both wound-healing assay and local invasion detection suggested that the combination could inhibit prostate cancer metastasis. The molecular profiling of gene expression of the xenografts using microarray indicated that the metastasis/migration/invasion functions were inhibited by the combination shown in IPA. And the combination could highly improve prostate cancer patient clinical outcome and reduce metastatic event with Oncomine analysis.

Conclusions: The current study, obtained with a highly clinically relevant prostate cancer model, suggest, for the first time, that docetaxel-based therapy of advanced human prostate cancer may be improved by combining docetaxel with Aneustat. Moreover, the combination could inhibit prostate cancer metastasis.

Outcome/Impact: Our study indicates that the prognosis of prostate cancer patients could be efficiently improved by the combination.
ELUCIDATING THE ONCOGENIC NETWORK THAT CO-OPERATES WITH PTEN LOSS IN BREAST CANCER

Wang, S.1, J. Liu2, G. Bader3, C. Pellecchia3, E. Zacksenhaus1,2

1Department of Laboratory Medicine and Pathobiology, University of Toronto, 2Toronto General Research Institute, University Health Network, 3Donnelly Centre for Cellular and Biomolecular Research

Introduction: Loss of the tumour suppressors PTEN is associated with aggressive triple negative breast cancer (TNBC), yet the oncogenic signaling that co-operate with PTEN loss are poorly understood. To elucidate these pathways we interrogated tumour initiating cells (TICs) in mammary tumours from PTENnull mice.

Methods: Mammary tumours from WAP-Cre:Ptentff mice were analyzed by histology, marker expression, transplantation assays, array CGH and microarray analysis. Effects of a dominant negative allele of p53, p53LSL-R270H, were determined by genetic analysis and cluster analysis. Metastatic potential was assessed following intravenous injection. miR143/145 was knocked-down using a lenti-sponge.

Results: Ptennull mice gave rise to two major tumour subtypes: poorly differentiated (PD; 6.4%) and adenomyoepithelioma (AME; 93.6%). Whereas the PD tumours contained TICs, capable of inducing secondary tumours following transplantation into recipient mice with 100% efficiency, AMEs failed to seed secondary tumours. The PD tumours clustered with HER2 and basal-like tumours. Interestingly, PD tumours exhibited 7.4-fold reduced expression of mir145 relative to AME tumours, and knockdown of mir145 in AME tumour cells increased proliferation and migration in vitro. Effects in vivo and the regulatory network that controls miR145 are being investigated.

WAP-Cre:Ptentff:p53LSL-R270H double mutant mice developed 4 tumour types including spindle and PD, both containing high frequency of TICs. The spindle tumours clustered with human Claudin-low TNBC, whereas PD tumours clustered with basal-like TNBC. WAP-Cre:Ptentff:p53LSL-R270H tumour cells were more metastatic than WAP-Cre:Ptentff:p531/2 double deletion tumours. Effect of p53 status on sensitivity of these nearly identical tumours to chemotherapy is ongoing.

Conclusions: We developed two mouse models for basal-like breast cancer that depend on miR143/145 expression or on p53 mutation.

Outcome/Impact: Deletion of Pten induces benign or aggressive tumours depending on cooperating oncogenic events. As these cooperating oncogenic networks are targetable, their inhibition may synergies with PI3K antagonists to suppress growth of PTEN-deficient BC.
59. METFORMIN ANTAGONIZES CANCER CELL GROWTH BY SUPPRESSING MITOCHONDRIAL-DEPENDENT MACROMOLECULAR BIOSYNTHESIS

Griss, Takla¹,², Faubert Brandon¹,², Jones Russell G.¹,²

¹Goodman Cancer Research Centre, ²Department of Physiology, McGill University

Introduction: Metformin, an electron transport chain (ETC) complex I inhibitor, is widely prescribed for treatment of type II diabetes and has been shown to decrease cancer burden in diabetic patients. However, the mechanism by which metformin impairs cancer cell proliferation remains unknown.

Methods: Crystal violet incorporation assays and cell counts were used for proliferation measurements. Gas chromatography coupled to mass spectrometry was used to assess intracellular metabolite levels and fatty acid levels. Seahorse bioanalyzer and NOVA bioprofiler was used to examine extracellular milieu metabolite levels.

Results: We observed increases in glucose uptake and lactate production in cancer cells treated with metformin. Real-time measurements of mitochondrial and glycolytic activity in cells show that metformin treatment causes a significant decrease in oxygen consumption combined with an increase in the extra cellular acidification rate. These effects were independent of metabolic checkpoints such as AMPK, LKB1, and mTOR. Further, treatment of osteosarcoma cells lacking a functional complex III of the ETC with metformin revealed that cells lacking a functional ETC were resistant to metformin’s anti-proliferative effects. Lastly, as a consequence of reduced TCA metabolites following metformin treatment, inhibition of fatty acid synthesis is observed, resulting in lowered biomass availability required for proliferation. siRNA treatment of ATP-citrate lyase resulted in reduced potency of metformin treatment, suggesting further that fatty acid synthesis is downstream of metformin’s anti-proliferative effects.

Conclusions: In this study, we highlight novel mechanisms by which metformin impairs cancer cell proliferation and demonstrate the importance of the metabolic effects in its anti-proliferative properties. We also show that these anti-proliferative effects were independent of classical metabolic checkpoints such as AMPK, LKB1, and mTOR. Finally, the importance of a functional ETC is crucial for all the effects of metformin, both metabolic and anti-proliferative.

Outcome/Impact: These results highlight the importance of a functional ETC for the effects of metformin. This knowledge could direct future therapies by highlighting tumours more dependent on mitochondria for energetic and anabolic purposes. These tumours would be more susceptible to metformin treatment compared to cancer cells that rely more on alternate pathways to meet their energetic and biomass needs.
60. POST-BISULFITE ADAPTER LIGATION: A HIGH-THROUGHPUT METHOD FOR SINGLE-CELL WHOLE-GENOME METHYLATION SEQUENCING

Hui, Z.K.¹, Heravi-Moussavi A², Laks E³, Mingay M¹, Moksa M¹, Aparicio S³,⁴, Hirst M¹,²

¹Department of Microbiology and Immunology and Centre for High-Throughput Biology, UBC, Vancouver; ²Canada’s Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver; ³Department of Molecular Oncology, BC Cancer Agency, Vancouver; ⁴Department of Pathology and Laboratory Medicine, UBC, Vancouver

Introduction: Genomic instability within cancer cells often leads to profound genomic heterogeneity, clonal evolution, and drug resistance. We hypothesize that epigenomic heterogeneity should exist within tumour cell populations to at least the same degree. Whole-genome bisulfite sequencing is a technique used to profile DNA methylation; however, this methodology is limited by high input DNA requirements (at least 100s of thousands of cells). We therefore aimed to develop a bisulfite sequencing method that can profile single-cells for the purpose of characterizing the epigenomic heterogeneity within tumour cell populations.

Methods: We developed a method, termed Post-Bisulfite Adapter Ligation (PBAL), which combines automated bisulfite conversion of DNA with traditional whole-genome shotgun library construction; this protocol can be scaled with an Agilent Bravo liquid handling robot, allowing for interrogation of many individual single-cells in parallel. Completed libraries are indexed, pooled, and sequenced on the Illumina massively parallel sequencing platform. Reads are then aligned to the human genome using a specialized bisulfite sequencing aligner (Novoalign).

Results: To date, we have sequenced 889 PBAL libraries on the Illumina MiSeq and 148 on the Illumina HiSeq. Using a filter within Novoalign to remove contaminants, we averaged <1% alignment from negative controls, 30% alignment from single-cells, and 85% alignment from control populations of 100 cells. The conversion efficiency of unmethylated cytosines measured, using a T7 control genome, was 98.5%. Analyses of 168 breast epithelial single cells (184-hTert) revealed an average of ~20% difference in CpG methylation between pairwise common CpG’s. Finally, we were able to detect copy-number variations and are currently working to associate these with epigenetic changes.

Conclusions: We have successfully developed a protocol for whole-genome DNA methylation using single-cells as input. We applied this protocol to assess epigenetic heterogeneity within 184-hTert immortalized breast epithelial cells and found ~20% heterogeneity at a single CpG resolution.
**61. STRUCTURE-BASED STUDY TO OVERCOME CROSS-REACTIVITY OF NOVEL ANDROGEN RECEPTOR INHIBITORS**

Li, Huifang¹, Nada Lallous¹, Kush Dalal¹, Eric Leblanc¹, Fuqiang Ban¹, Fabrice Ciesielski², Bonny Chow¹, Helene Morin¹, Kriti Singh¹, Paul S. Rennie¹ and Artem Cherkasov¹

¹Vancouver Prostate Centre, University of British Columbia; ²NovAliX, BioParc, 850 bld Sébastien Brant, F-67405 Illkirch, France

**Introduction:** The mutation-driven transformation of clinically used antiandrogens into agonists of human androgen receptor (AR) represents a major challenge in the current treatment of prostate cancer (PCa).

**Methods:** To address this problem, we have developed a novel class of AR inhibitors targeting the DNA-binding domain (DBD) of the receptor, which is distanced from the mutation-prone androgen binding site (ABS) in the ligand-binding domain (LBD) of the AR, targeted by all conventional antiandrogens.

**Results:** While many members of the developed phenyl-thiazol-2-yl-morpholine series demonstrated potent sub-micromolar inhibition of the wild-type AR, some compounds also exhibited an undesired partial agonistic effect toward the T877A mutated form of the receptor, implying their cross-interaction with the AR ABS. To study the molecular basis of the cross-reactivity, we have solved the T877A mutated form of the AR LBD in complex with one developed compound exhibiting such unwanted partial agonism. Based on the resolved crystal structure, we have identified critical protein-ligand interactions and conformational changes in wild-type and T877A forms of AR that drive observed agonistic effects.

**Conclusions:** The identified structural basis has further been used to modify the scaffold of developed AR DBD binders to eliminate their cross-reactivity toward T877A mutated AR LBD. In particular, the replacement of the phenyl ring with less hydrophobic heterocycles resulted in a series of inhibitors that did not demonstrate affinity toward ABS or exhibit an undesired agonistic effect on AR.

**Outcome/Impact:** This study provides insights into the development of AR inhibitors with high specificity, which may help overcome the mutation-driven resistance of antiandrogens in the treatment of advanced PCa.
62. DYNAMIC TRANSCRIPTOME ANALYSIS OF DNA DAMAGE PATHWAYS UNDER HYPOXIA

Lo, Winnie W.¹, Gaetano Zafarana¹, Robert G. Bristow¹,²

¹Radiation Medicine Program, Princess Margaret Cancer Centre, University Health Network, Toronto; ²Departments of Radiation Oncology and Medical Biophysics, University of Toronto

Introduction: The RNA levels of DNA repair pathway genes have been shown to be down-regulated in hypoxia; however, most of the RNA studies do not distinguish between RNA synthesis and degradation. Here, we use novel metabolic labeling of newly synthesized RNA to investigate the dynamic changes in transcription rates of DNA repair pathways of cells under hypoxia.

Methods: 4-thiouridine (4sU), a uridine analogue, was added to the media of DU145 cells (prostate cancer cell line) that have been cultured in 21% and 0.2% O₂ for 72 hours. Total RNA was extracted and 4sU-tagged RNA was biotinylated and separated from untagged pre-existing RNA using streptavidin coated magnetic beads. Real-time RT-PCR was used to quantify the expression of repair pathways in three RNA subsets (total, pre-existing, and newly synthesized). Parallel experiments were performed in RNA harvested from the cytoplasmic and nuclear factions of cells.

Results: We found that with 30-minute 4sU incubation, most tagged RNA is still in the nucleus, and is subjected to little degradation, thus reflecting the average transcription rate. The percentage of total 4sU uptake is lower in hypoxic cells. Importantly, the expression of homologous recombination (HR) repair pathway regulators, RAD51, BRCA1 and BRCA2, is decreased in both total and newly-synthesized RNA of hypoxic cells.

Conclusions: Our results demonstrate that in addition to translational regulation, hypoxia may also downregulate repair pathways through suppression of transcription. This 4sU assay provides opportunities for further analysis of differential RNA synthesis and decay of DNA repair pathways between hypoxia and normoxia.

Outcome/Impact: Intratumoural hypoxia is a prognostic factor associated with decreased disease-free survival and increased resistance to radiotherapy and chemotherapy in cancers. By understanding the mechanisms behind how hypoxia alters the expression of genes and pathways, we can incorporate treatment strategies that target otherwise resistant hypoxic cells to improve prognosis.
63. EVALUATING CLONAL HETEROGENEITY AND TUMOUR EVOLUTION IN DIFFUSE LARGE B-CELL LYMPHOMAS

Mohajeri, Arezoo¹, Jasleen Grewal¹, Miguel Alcaide¹, Sarit Assouline³, Koren Mann³, Nathalie Johnson³, Ryan D. Morin¹,²

¹Department of Molecular Biology and Biochemistry, Simon Fraser University; ²Genome Sciences Centre, BC Cancer Agency; ³Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University.

Introduction: Some patients with Diffuse Large B-cell Lymphoma (DLBCL) do not respond or relapse after standard therapies. We are using global and targeted next-generation sequencing to detect mutations and infer patterns of clonal evolution between primary (T1) and relapse (T2) tumour to identify mechanisms involved in relapse.

Methods: Whole exome sequencing was performed on T1 samples and matched normal DNA to detect somatic point mutations and indels. Based on recurrence and our prior work, mutations suspected to act as drivers were selected for further experiments. Specifically, targeted amplicon and hybridization-capture sequencing were performed on the T2 DNA samples. Subsequently the allelic fractions of mutations were compared between T1 to T2 samples, looking for changes in allelic frequency of mutations between the two time points, which is an indication of clonal evolution. We also calculated purity of tumour samples and ploidy of cancer cells to estimate the fractions of cancer cells bearing each mutation.

Results: Comparison between allelic frequency of selected mutations in primary and relapse tumour samples suggest presence of clonal evolution in 6 of tumour samples. We observed increase and decrease of VAF in 10 and 2 genes, respectively. Cancer cell fractions with the mutations in genes: FOXO1, MLL2, TNFRSF14, CREBBP, STAT6, GNAI2, RB1, CD79B, TP53 and MLL3 were increased in relapsed tumours.

Conclusions: We detected increases in frequency in relapsed tumours of mutations in 10 genes, which is an indication of clonal expansion of cancer cells carrying these mutations. Detection of such mutations and, in particular, any genes for which mutations are consistently enriched on the path to relapse, will provide us more information on causes of resistance to therapy and subsequent relapse.

Outcome/Impact: Genetic heterogeneity and clonal evolution in DLBCLs are likely to contribute to treatment resistance in DLBCL. Studying the genetic patterns of mutations in relapse for these patients can help in their treatment. Detecting the pattern of clonal evolution in DLBCLs is an important step toward identifying therapeutic options for patients whose tumours acquire resistance to standard treatments.
64. REGULATION OF TRANSLATION IN KAPOSI’S SARCOMA

Pringle, Eric S.1,2, Andrew M. Leidal3, Carolyn-Ann Robinson1, James Uniacke4, Craig McCormick1,2

1Department of Microbiology and Immunology, Dalhousie University. 2Beatrice Hunter Cancer Research Institute. 3Department of Pathology, University of California, San Francisco. 4Department of Molecular and Cellular Biology, University of Guelph.

Introduction: Kaposi’s sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi’s sarcoma (KS), the leading AIDS-related malignancy. Here we analysed the requirements of mTORC1 activation during KSHV replication.

Methods: We use a KSHV lytic replication cell culture model system and pharmacological inhibition of mTORC1. Translation analysis was performed using polysome profile analysis, 7m’GTP pull-downs, and stable isotope labelling quantitative mass spectrometry.

Results: We found that mTORC1 activity was required for efficient reactivation from virus latency, but dispensable for genome replication and release of progeny virions. During KSHV lytic reactivation, the mTORC1-4EBP1-eIF4F axis was intact, yet KSHV gene products were efficiently translated when eIF4F was disassembled through mTORC1 inhibition. We identified several viral gene products associating with 7’mGTP beads during lytic replication.

Conclusions: mTORC1 inhibition is known to contribute to KS regression, but the requirement of mTORC1 activation for translation of KSHV gene products is unclear. Here we demonstrate that translation of viral gene products, sufficient for virion production, are resistant to mTORC1 inhibition.

Outcome/Impact: Viruses require the host translation machinery, and are therefore threatened by host mechanisms that evolved to sense infection and arrest translation. Our studies will reveal new viral mechanisms for gaining control of the translation machinery.
65. REFINEMENT AND ALIGNMENT: COMBINED GENETIC MAPPING AND WHOLE LOCUS SEQUENCING FOR THE GRANULOSA CELL TUMOUR SUSCEPTIBILITY 1 LOCUS IN SWR MICE

Smith, Kerri¹, Yaskowiak Edward¹, Beamer Wesley², Reinholdt Laura², Dorward Ann¹

¹Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John’s, NL; ²The Jackson Laboratory, Bar Harbor, Maine

Introduction: Female mice of the SWR/Bm (SWR; allele SWR) inbred strain are susceptible to the spontaneous initiation of ovarian granulosa cell (GC) tumours at puberty. This heritable trait is unique to the SWR strain, implying genetic variation at one or more loci in the SWR genome dictates GC tumour susceptibility. Furthermore, the number of females that develop GC tumours is greatly increased with androgen treatment (dehydroepiandrostosterone (DHEA) or testosterone) at the pubertal transition. Forward genetic mapping strategies have confirmed strong linkage between GC tumour susceptibility and a DHEA-responsive locus on distal Chr 4 named granulosa cell tumour susceptibility 1 (Gct1SWR). Phenotype-driven mapping using subcongenic lines incorporating genome from the tumour-resistant Castaneus strain has narrowed the Gct1 interval to 1.31 Mb and 17 annotated genes. We hypothesize that Gct1SWR is a regulatory genetic polymorphism that supports an increased probability of GC tumour initiation following normal endocrinological stimulation of the maturing mouse ovary.

Methods: Whole-locus targeted capture and Next Generation sequencing technologies were combined to identify Gct1 polymorphisms unique to SWR. Common polymorphisms were eliminated by in silico comparison to genome sequences from tumour-resistant strains. Of over 2,000 predicted novel variants, a subset was prioritized for independent verification by Sanger sequencing.

Results: Confirmed regulatory variants in four genes – Dhrs3, Tnfrsf1b, Tnfrsf8, and Vps13d – were prioritized for further investigation of their impact on gene expression and protein function. A significant variant under investigation is a putative splice site mutation in the Dhrs3 gene, a short-chain dehydrogenase/reductase involved in retinoic acid metabolism.

Conclusions: The combined strategies of forward mapping and high throughput sequencing have prioritized regulatory variants in the Gct1SWR locus that are relevant to endocrine-sensitive GC tumour initiation in pubertal SWR female mice.

Outcome/Impact: The identification of a DHEA-specific ovarian GC tumour susceptibility gene will have implications for human, juvenile-onset GC tumours, as well as for other ovarian pathologies (e.g. epithelial ovarian cancer, polycystic ovary syndrome). Furthermore, the elucidation of Gct1 will potentially introduce molecular diagnostic tumour sub-classification criteria which is severely lacking for this tumour type.
DISTINGUISHING BETWEEN PASSENGER AND MAINTENANCE GENETIC EVENTS IN MEDULLOBLASTOMA THROUGH A NOVEL SLEEPING BEAUTY AND PIGGYBAC NESTED TRANSPOSON SCREEN

Wang, Xin¹, John Peacock¹, Juan Cadinanos³, Adam Dupuy⁴, Marc Remke¹, Vijay Ramaswamy¹, Stephen Mack¹, Allan Bradley³, Michael D. Taylor¹,²

¹Department of Laboratory Medicine and Pathobiology, ²Hospital for Sick Children, University of Toronto, Toronto, ON, Canada, ³Welcome Trust Sanger Institute, Cambridge, United Kingdom, ⁴Molecular and Cell Biology Program, University of Iowa, Iowa City, IA, USA

Introduction: Medulloblastoma is the most common malignant paediatric brain tumour. Medulloblastoma pathogenesis is poorly understood, and, of grievous importance, there are no currently approved targeted therapies.

Methods: The Sleeping Beauty (SB) and piggyBac (PB) transposon systems were shown to be effective tools for functional genomics studies of solid tumour initiation and progression. The transposon is engineered with both loss-of-function and gain-of-function elements to inactivate tumour suppressors or activate oncogenes at its insertion site, thereby promoting tumour formation. We have designed a novel SB and PB nested transposon to identify maintenance events in medulloblastoma using an insertional mutagenesis screen. Transgenic mice expressing the nested transposon, Lazy Piggy (LP), SB transposase (Nestin:Luc-SB100), and an inducible PB transposase (Rosa:LSL-mPB-Ert2) were generated.

Results: To date, we have double transgenic mice expressing both the LP transposon and Nestin:Luc-SB100. Furthermore, we have crossed these mice to a Ptc¹/² background to increase tumour incidence. Together, we are able to produce a highly penetrant model of medulloblasoma. These tumours are reminiscent of their human counterpart via histology and molecular profiling. This is the first demonstration that a SB and PB nested transposon is able to recapitulate human medulloblastoma. We are currently in the process of sequencing the resultant tumours to identify the gCISs (gene-centric common insertion sites). Preliminary experiments have shown successful mobilization of the LP transposon after tamoxifen induction in vitro.

Conclusions: Mobilizing SB transposition in Nestin expressing cells generated a highly penetrant model of medulloblastoma. Activating PB transposition through temporal regulation gradually depleted passenger events while enriching for shared maintenance genes (SMG); ongoing gCIS analysis will identify the SMGs.

Outcome/Impact: This model will allow us to discriminate shared maintenance genes from initiation and passenger events, which will ultimately increase our understanding of the disease and reveal potential therapeutic targets. Successful application of this novel model system can be applied to the study of other types of cancer.
DECONVOLVING TRANSCRIPTIONAL SIGNATURES OF HYPOXIA, STROMA, AND TUMOUR AGGRESSIVITY

Anghel, Catalina V.1, Lalonde Emilie1,2, Deshwar Amit1, Sykes Jenna6, Pintilie Melania6, Bristow Robert2,4,6, Boutros, Paul C.1,2,5

1Ontario Institute for Cancer Research, Toronto, ON; Departments of 2Medical Biophysics, 3Electrical & Computer Engineering, 4Radiation Oncology, and 5Pharmacology & Toxicology, University of Toronto, Toronto, ON; 6Princess Margaret Cancer Centre, Toronto, ON

Introduction: Hypoxia has been associated with increased cancer recurrence, metastasis, and decreased response to radiotherapy in patients. Distinguishing mRNA abundance profiles associated with hypoxia and other micro-environmental factors from the bulk tumour profile of each patient is essential to characterize the heterogeneity of the tumour and develop accurate, clinically-useful biomarkers.

Methods: Two genomic datasets of treatment-naïve cervix (72 patients) and prostate cancer (124 patients) with corresponding measurements of tumour oxygen pressure were analyzed. A hierarchical Bayesian model will be used for the deconvolution, building on a validated model which estimates tumour cellularity and cancer subtype. The correlation of hypoxia and other measured micro-environmental characteristics with the proportions of inferred sub-populations will allow us to evaluate the accuracy of the model.

Results: While hypoxia alone is not a significant prognostic biomarker in either dataset, combining hypoxia with genetic instability showed a multiplicative and independent prognostic effect in the prostate cancer dataset (Interaction Hazard Ratio 3.8, 95% Confidence Interval, Wald p=0.013). For the cervix cancer dataset, previous studies have shown a slight prognostic effect of hypoxia for patients with node-negative cervix cancer. Preliminary work on dimension reduction on the cervix cancer dataset using principal component analysis and k-means clustering does not show clear clusters. Instead, a hierarchical Bayesian model with an infinite mixture of Dirichlet distributions as the prior on the inferred cancer profile would allow the number of clusters to be learned from the data (incorporating the Chinese Restaurant Process to favour fewer clusters). We will then select the most likely clusters and determine the decomposition of the cancer component with respect to these profiles.

Conclusions: The interaction of hypoxia with other indices in prognostic prediction demonstrates the importance of tumour micro-environment on patient outcome. The deconvolution of mixed tumour into distinct micro-environmental profiles is an important prerequisite in modelling outcome based on both micro-environmental and molecular data.

Outcome/Impact: The characterization of sub-populations related to tumour micro-environment is valuable for improving the accuracy of prognostic biomarkers and targeting treatment.
68. LOSS OF Estrogen-Related Receptor Gamma is REQUIRED FOR Androgens-Mediated Energy Reprogramming of Prostate Cancer Cells

Audet-Walsh, Etienne, Tracey Yee, Ming Yan, Mathieu Vernier, Ryan Butler, Carlo Ouellet, Armin Pause, and Vincent Giguère

Goodman Cancer Research Centre, McGill University, Montréal

Introduction: Androgen signalling is known to be central to prostate cancer (PCa) development. Energy metabolism reprogramming is now considered a hallmark of cancer, but a clear understanding of how androgens modulate it is still required. As the estrogen-related receptor gamma (ERRγ) is known to be a master regulator of energy metabolism, we investigated its interplay with the androgens signalling.

Method: Chromatin immunoprecipitation (ChIP) coupled to deep sequencing (ChIP-seq) or to quantitative PCR (ChIP-qPCR) were employed to validate protein binding to target genes. qRT-PCR and microarray analysis were employed to validate transcriptional regulation following siRNA experiments or hormonal treatments in prostate cancer cells in vitro and in vivo. Several metabolomics tool, including gas chromatography coupled to mass spectrometry (GC-MS), were finally employed to assess the biological consequences of altered transcriptional regulation.

Results: We identified ERRγ as a novel negative target of the androgen receptor (AR), in vitro in PCa cells and in vivo in healthy mouse tissues. We also showed that ERRγ was essential for mRNA modulation of several metabolic target genes upon androgens treatment. Importantly, following androgen stimulation, glucose is used to fulfill high aerobic glycolysis, while the mitochondrial metabolic profile shifts, relying almost completely on fatty acids beta-oxidation. By modulating ERRγ activity through RNA interference and overexpression systems, we showed that ERRγ positively regulates glutamine metabolism and its knockdown was required for the switch from glutamine to glucose usage induced by androgens. Moreover, by investigating ERRγ expression in several clinical studies, we revealed that its expression is significantly decreased in PCa metastasis and in recurrent diseases.

Conclusion: We identified a novel mechanism by which androgens, through the regulation of ERRγ, controls prostate cancer cell metabolism.

Outcome: We identified ERRγ as an important factor for prostate cancer cell metabolism and as a potential novel therapeutic target of prostate cancer.
69. ROLE OF S100A10 (p11) IN THE PATHOGENESIS OF BREAST CANCER: AN INSIGHT USING TRANSGENIC MOUSE TUMOUR MODEL

Bharadwaj, Alamelu1, Ryan Holloway2, Paul O’ Connell2, David Waisman1,2

1Department of Biochemistry and Molecular Biology, 2Department of Pathology, Dalhousie University, Halifax, NS, Canada

Introduction: The complex cascade of metastasis in breast cancer progression is largely dependent on the degradation of extracellular matrix proteins by tumour and stromal cell proteolytic enzymes such as plasmin. Generation of plasmin from plasminogen is accelerated by plasminogen receptors such as p11. Our studies are focussed on understanding the function of p11 in breast cancer pathogenesis with an aim to identify new diagnostic tools and effective interventions in breast cancer therapy.

Methods: We employed a transgenic mouse model for breast cancer, Polyoma Middle T (PyMT), which spontaneously develops mammary tumours and pulmonary metastases driven by the PyMT oncoprotein. We generated mice that express PyMT in a p11-wild type (PyMT/p11+/+) and p11-null background (PyMT/p11-/-), and monitored the tumour growth in the mice every week. We also harvested and stained the abdominal mammary glands at 4, 6, 8, 10, 12 weeks of age to identify histopathological stages of tumour progression. At the end-point (4000 mm³ total tumour volume), the mice were sacrificed and total tumour burden and tumour weight, were determined. Metastasis in PyMT/p11+/+ mice and PyMT/p11-/- mice were evaluated by both macroscopic and microscopic examination of the (hematoxylin and eosin staining) lungs.

Results: Our initial studies have demonstrated that there is impairment of tumour growth rate in the PyMT/p11-null mice. Hyperplastic lesions appear in the mammary gland of PyMT/p11-WT mice as early as 8 weeks of age, whereas very sparse lesions are observed in the PyMT/p11-null mice. We also observed a 40% reduction in pulmonary metastatic incidence.

Conclusions: We have successfully established the PyMT mouse mammary transgenic model which recapitulates the human breast cancer disease progression and identified an important role for p11 in mammary tumour growth and metastasis.

Outcome/Impact: The critical challenge that we encounter in the treatment and high mortality of breast cancer patients is associated with the increased incidence of metastasis. Our ongoing studies propose an important link between p11 and cancer cell invasion and metastasis, with a potential of developing into a therapeutic target.
70. RESPONSE MONITORING USING TEXTON-BASED APPROACH IN LOCALLY ADVANCED BREAST CANCER

Gangeh, Mehrdad J.1, Hadi Tadayyon1, Lakshmanan Sannachi1, Ali Sadeghi-Naini1, Maureen Trudeau2, Sonal Gandhi2, and Gregory J. Czarnota1

1Physical Sciences, Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, Toronto; 2Division of Medical and Haematologic Oncology, Department of Medicine, Sunnybrook Health Sciences Centre, Toronto

Introduction: Assessing the efficacy of cancer treatments in preclinical and clinical treatments is presently limited; results may not be available to the clinician for typically months. Quantitative ultrasound (QUS) methods provide a promising framework that can non-invasively, inexpensively, and quickly assess tumour response to cancer treatments using standard ultrasound equipment.

Methods: Fifty six patients with locally advanced breast cancer (LABC) who received neoadjuvant chemotherapy were imaged before and at 4 times during treatment, i.e., weeks 1, 4, 8 and pre-operatively. Mid-band fit parametric maps were computed by deploying quantitative ultrasound spectroscopy techniques. The patients were grouped into responding (R) or non-responding (NR) based on their ultimate clinical and pathological response to treatment. Codebooks of textons were constructed for each patient. Subsequently, a histogram of textons was computed for each parametric map using the associated codebook as the model to represent the pre- and during-treatment images. The distance between these features for each subject was used as a criterion of the effectiveness of the treatment, which was ultimately submitted to a naïve Bayes classifier to classify the patients to R/NR in a leave-one-subject-out manner.

Results: The classification of patients with LABC to R/NR using the proposed texton-based system achieved an accuracy of 84% and 85%, area under curve (AUC) of 80% and 83%, sensitivity of 87% and 86%, and specificity of 81% and 84% after 4 and 8 weeks of treatment, respectively.

Conclusion: In this study, texture methods based on texton-based approach was proposed to quantify the assessment of LABC response to neoadjuvant chemotherapy. The proposed system achieves a promising accuracy and sensitivity 4 weeks after treatment initiation.

Outcome/Impact: The framework presented in this study can facilitate non-invasive detection of refractory responses in patients to a certain cancer treatment early on during course of therapy to enable switching to more efficacious treatments. The results of this study would permit clinicians to receive feedback and switch to alternate treatments far earlier, in a step towards the goals of personalized medicine.
71. KINASE INHIBITORS AND BIGUANIDES AS A SYNTHETIC LETHAL APPROACH TO TREAT CANCER

Hulea, Laura1, Simon-Pierre Gravel2, Young Kyuen Im1, Yunhua Zhao1, Josie Ursini-Siegel1, Julie St-Pierre2, Michael Pollak1, Ivan Topisirovic1

1Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University, 2Goodman Cancer Research Centre, McGill University, Montreal, Quebec, Canada

Introduction: Biguanides (metformin, phenformin) are energy stressors that inhibit mitochondrial complex I, causing a compensatory increase in glycolysis. Importantly, metformin use was associated with lower cancer risk and better cancer prognosis. Both in the clinic and in the lab, tyrosine kinase inhibitors (TKIs) reduce tumour glucose uptake and glycolysis in human tumours and in tumour cell lines of various cancer types. In contrast, TKI (e.g. imatinib) resistant tumour cell lines maintain a highly glycolytic metabolic phenotype with elevated glucose uptake and lactate production. We tested whether TKIs lowering glycolysis, combined with biguanides, provide a synthetic lethal approach to treating various types of cancer and aimed to unravel the underlying mechanisms.

Methods: In vitro studies were performed on three cancer cell line models of i) HER2-transformed mammary gland cells (NMuMG-NT2197); ii) BRAFV600E positive melanoma (A375 cells) and iii) BCR-ABL positive CML (K562 cells), measuring proliferation, various metabolic parameters and status of signaling pathways, following individual or in combination treatments of phenformin and TKIs (i) lapatinib; ii)PLX4032; iii) imatinib, respectively). In vivo, NMuMG-NT2197 tumour growth following individual or in combination treatments of phenformin and lapatinib was measured.

Results: Combination of lapatinib with phenformin exhibited synergistic anti-proliferative effects in NMuMG–NT2197 cells in vitro. In vivo, the combination suppressed growth of NMuMG–NT2197 tumours to a higher extent than each drug alone. These anti-tumourigenic effects were accompanied by mTOR inhibition and AMPK activation. Phenformin suppressed OXPHOS, leading to a compensatory increase in glycolysis and glutamine-dependent reversal of the citric acid cycle (i.e. reductive glutamine metabolism), whilst lapatinib impaired this compensatory effect. Consistently, in A375 and K562 cells, combination of PLX4032 or imatinib, respectively, with phenformin had a synergistic anti-proliferative effect in vitro, which was paralleled by mTOR inhibition. Similarly to lapatinib, both PLX4032 and imatinib inhibited glycolysis, whilst treatment in combination with phenformin stopped this inhibition.

Conclusions: These findings indicate that clinically used TKIs impede adaptation to energy stress induced by suppression of mitochondrial function by biguanides, resulting in synergistic anti-proliferative and anti-neoplastic effects.
72. OXYGEN-INDEPENDENT DISULFIDE BOND FORMATION IN HYPOXIA INDUCED PROTEINS SUPPORTS THEIR EXPRESSION DURING HYPOXIA

Levitin, Fiana, Ryan Rumantir, Jenna Sykes and Marianne Koritzinsky

Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Introduction: Poor oxygenation in tumours lead to transcriptional up-regulation of a large number of genes such as carbonic anhydrase 9 (CA9) and vascular endothelial growth factor (VEGF). CA9 and VEGF maturation include introduction of intra-molecular and inter-molecular disulfide bonds in the endoplasmic reticulum (ER). Our data indicated that oxygen is strictly required for disulfide isomerization during disulfide bond formation. This results in severely reduced expression of proteins in the extracellular space during hypoxia in contrast with increased cell surface expression of hypoxia-induced proteins. We hypothesized that hypoxia-induced proteins such as VEGF and CA9 possess a unique ability to mature in the absence of oxygen ensuring their functional cell surface expression and secretion under hypoxia.

Methods: To directly monitor disulfide bond formation in target proteins, we 35S-methionine labeled cells under normoxia or anoxia. Following various chase periods, the protein of interest was immunoprecipitated and disulfide bond formation assessed on non-reducing SDS-PAGE gels. To address on a global scale whether hypoxia-induced proteins may have evolved traits that allow them to mature in hypoxia, we identified 113 extracellular proteins that were >2-fold expressed in hypoxia and 313 extracellular proteins that were <1.2 fold expressed in hypoxia from our microarrays of HCT116 and HepG2 cell lines.

Results: Our data demonstrate that disulfide bonds are introduced in CA9 and VEGF in the complete absence of oxygen. Interestingly, VEGF is matured and secreted faster under anoxic than normoxic conditions. In general, hypoxia induced proteins had significantly higher content of cysteines and disulfide bonds than non-induced proteins. Furthermore, a higher fraction of cysteines participated in disulfide bonds, and the disulfide bonds were of significantly shorter range.

Conclusions: Our data are consistent with a model where hypoxia-induced proteins have evolved to mature in the absence of oxygen through a low requirement for oxygen-dependent disulfide isomerization. ER-localized protein maturation likely represents a unique mechanism contributing to differential protein expression in the extracellular space during hypoxia.
73. CLINICAL STUDY OF EX VIVO PHOTOACOUSTIC IMAGING IN ENDOSCOPIc MUCOSAL RESECTION TISSUES

Lim, Liang1, Catherine J. Streutker2, Norman E. Marcon1, Maria Cirocco2, Vladimir V. Iakovlev2, Ralph Dacosta1, F. Stuart Foster3, Brian C. Wilson1

1Princess Margaret Cancer Centre, University Health Network, Toronto; 2St. Michael’s Hospital, Toronto; 3Sunnybrook Health Sciences Centre, Toronto

Introduction: Accurate endoscopic detection and dysplasia in patients with Barrett’s esophagus (BE) remains a major unmet clinical need. Current diagnosis use multiple biopsies under endoscopic image guidance, where up to 99% of the tissue remains unsampled, leading to significant risk of missing dysplasia. We conducted an ex vivo clinical trial using photoacoustic imaging (PAI) in patients undergoing endoscopic mucosal resection (EMR) with known high-grade dysplasia. Photoacoustic imaging (PAI) is an imaging modality that is sensitive to absorbers (e.g. blood), capable to image non-invasively deep into human tissues. The purpose of this project is to perform a feasibility study of acquiring photoacoustic signals from esophageal tissues for the purpose of characterizing the esophageal microvascular pattern.

Methods: EMR tissues taken immediately from the patient were mounted using ultrasound gel on an agar base layer. Digital photography guided the placement of the PAI transducer, which operated at 40 MHz and scanned the luminal side of the specimen in 14 mm wide strips at 680, 750, 824, 850 and 970 nm. Acoustic images were simultaneously acquired using a commercial PAI system (Vevo LAZR, Visualsonics, Toronto, Canada). Tissues were then sliced and fixed in formalin for histopathology. Ongoing analysis includes co-registration and correlation between the intrinsic PAI features and the histological images.

Results: PAI images map the blood distribution within the superficial layers of the scanned ex vivo tissue. We observed good co-registration between PAI+US images and histopathology.

Conclusions: Preliminary PAI+ultrasound images have demonstrated the technical feasibility of this approach and point to the potential of PAI to reveal the microvascular pattern within the EMR specimens.

Outcome/Impact: Results from this study confirm the feasibility and strengthen the potential of performing endoscopic PAI for non-invasive in vivo dysplasia detection. Future projects include application of targeting nanoparticles (porphysomes) as therapeutic guidance.
74. INDUCIBLE LENTIVIRAL EXPRESSION OF MICRONAS AS A STRATEGY TO OVERCOME THE DIFFERENTIAION BLOCK IN ACUTE MYELOID LEUKEMIA

Ruschmann, Jens1*, Tobias Maetzig1*, Kathrin Krowiorz2*, Florian Kuchenbauer2 and R. Keith Humphries1

1Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada V5Z 1L3; 2Internal Medicine III, University Hospital of Ulm, Germany

Introduction: As part of the TFRI MDS/AML Research Consortium we recently established a high-throughput microfluidic real-time quantitative PCR platform, and developed miRNA-based expression signatures from enriched malignant subpopulations purified from over 50 AML patients. We identified 25 miRNAs with significant differential expression between more mature leukemic cell populations (bulk, myeloid enriched and/or CD34-) and more primitive stem/progenitor leukemic populations (CD34+ and/or CD34+CD38-). Here focus on 2 such miRNAs, miR-150 and miR-193a, that are enriched in more mature, stem cell deplete, cell populations to test whether their engineered overexpression would induce differentiation and suppress leukemogenic activity in a murine leukemia model.

Method: We established a lentiviral doxycycline inducible expression system for miRNAs and assessed the impact of miRNA expression in a murine HoxA9/Meis1 (HA9M) leukemia model using a variety of in vitro and in vivo readouts.

Results: In transduced HA9M leukemic cells we achieved >100 fold increase of mature miRNA expression upon addition of doxycycline within 72h, as quantified by TaqMan PCR. Expression of miR-150 causes an >10-fold growth disadvantage and a 60% reduction of c-kit+ cells relative to un-induced HA9M leukemic cells in vitro, and an increase of the differentiation markers CD11b and Gr1 that was further enhanced in the presence of all trans retinoic acid or G-CSF. In vivo studies of HA9M with inducible miR-150 and miR-193a are in progress to examine directly their potential to suppress leukemogenic activity. Mice have been transplanted with miR-150 or miR-193a transduced cells. After 3 weeks miRNA expression will be induced with a doxycycline containing chow and changes in engraftment, differentiation markers and survival will be followed.

Conclusions: Leads from patient based gene expression profiling coupled to an inducible lentiviral vector system have provided preliminary evidence of the potent ability of selective miRNA expression to overcome the differentiation block in a murine leukemia model that may significantly suppress leukemogenic activity.

Outcome/Impact: The identification of miRNAs with potent pro-differentiation and anti-leukemic activity provides impetus to develop miRNAs as novel therapeutics and to exploit such miRNAs for further target identification.
75. MUTATION ANALYSIS OF CELL LINES DERIVED FROM MATCHED PRIMARY AND RECURRENT HIGH GRADE SEROUS EPITHELIAL OVARIAN CANCERS USING NEXT GENERATION SEQUENCING

Tchakarska, G.¹, E Carmona¹, SP Shah⁴, D Huntsman⁵, D Provencher¹,²,³, AM Mes-Masson¹,²

¹Centre de recherche du Centre hospitalier de l'Université de Montréal/Institut du cancer de Montréal; ²Department of Medicine and ³Department of Obstetrics-Gynaecology, Oncological Gynaecology Service, Université de Montréal; ⁴Department of Molecular Oncology and ⁵Department of Pathology and Laboratory Medicine, BC Cancer Agency, Vancouver

Introduction: Unlike other solid cancers, in high grade serous epithelial ovarian cancer (HGS EOC) there is only one gene, p53, which is mutated in the majority of the cases (80%). HGS EOC is characterized by a high rate of genomic instability displaying complex karyotypes, which may evolve during tumour progression suggesting a link between genomic instability and cancer progression. In this study we compared the mutational landscape in HGS EOC cell lines derived from the same patient, from the tumour at diagnosis and a second tumour, developed after treatment and recurrence.

Methods: The exome of nine cell lines, derived from three patients, 1369, 2295 and 3133, and the blood exome for patients 2295 and 3133 were sequenced using the exome capture Illumina technology. Mutation validation was done by Sanger sequencing. Chromosome number was determined by metaphase spreads.

Results: The nine cell lines contain 51 chromosomes on average, ranging from 45 to 90, which is a first demonstration of chromosomal instability in our experimental model. Exome capture sequencing identified in average 177 somatic mutations per cell line for patients 2295 and 3133. For patient 1369, 505 mutations per cell line, including possible polymorphisms and germinal mutations were detected. The validation rate was about 70%, as expected. Mutated gene function was determined and pre- and post-chemotherapy cell lines from the same patient were compared. The only mutated gene in common to all nine cell lines is p53, as expected.

Conclusions: Our experimental model is particularly pertinent for this study since it allows for the genetic follow up of the same patient during cancer progression. Karyotyping and exome sequencing results demonstrated the genetic heterogeneity characteristic for ovarian cancer. Outcome/Impact: We will establish the effect of pertinent mutations on protein function. This study may permit the identification of new genes involved in tumour progression and/or chemoresistance.
76. EARLIEST SITES OF HEPADNAVIRUS INTEGRATIONS INTO HOST GENOME AFTER DE NOVO INFECTION OF HEPATOcyTE-LIKE HEpARG CELLS AND WOODCHUCK LIVERS

Chauhan, Ranjit1, Norma D. Churchill1 and Thomas I. Michalak1

1Molecular Virology Group, Faculty of Medicine, MUN, Canada

Introduction: Hepatocellular carcinoma (HCC) is the 5th most common human cancer with dismal 5-year survival. Hepatitis B virus (HBV) is highly oncogenic and its DNA integration precedes HCC development. The sites where virus DNA initially integrates into the host's genome are not recognized. Woodchucks infected with woodchuck hepatitis virus (WHV) represent pathogenically most relevant model of human HBV-induced HCC.

Methods: Two exploratory systems were used: (1) human hepatocyte-like HepaRG cells prone to authentic HBV were investigated in 15min, 30min, 1h, 3h, 24h, 2d, 7d, 15d, 29d and 50d post-infection and (2) woodchucks infected with WHV from which liver biopsies were obtained at 1h or 3h and at 6wk post-infection. HepaRG cells were analyzed for HBV X gene integrations, while liver biopsies for WHV X and preS sequence integrations using inverse-PCR followed by cloning and sequencing to identify virus-host and virus-virus DNA junctions.

Results: Intracellular HBV DNA and its replication intermediates became detectable in HepaRG from 1h and remained until 50d post-exposure. HBV DNA integrations were apparent in 1h post-infection. However, within 1wk HBV DNA integrated at much higher magnitude at host’s LINE-2 element, followed by LINE-1, SINE and STA-II. In addition, HBV-host DNA integrations were found in satellite III DNA sequences and in genes playing roles in chromosomal translocations. Also, virus-virus DNA junctions were detected. In woodchucks, WHV mRNA, indicative of active replication, was detected in livers from 1h or 3h post-infection and, at the same time, WHV-host DNA integrations were identified. The HBV core promoter/enhancer II region and its WHV equivalent appeared to be predisposed to form the earliest virus-host DNA junctions.

Conclusion: HBV DNA surprisingly early integrates into the host’s cell DNA and possibly uses retrotransposons elements LINE-2, LINE-1, SINE and STA, and translocation genes for its spread and oncogenic perturbations. This predisposition to a very early integration into the host's genome was confirmed in WHV-infected woodchucks.

Outcome/Impact: Using in vitro and in vivo infection models, this study for the first time revealed the earliest time points at which hepadnaviral DNA integrate into host genome and possibly explain a mechanism by which these integrations spread across chromosomes.
77. TP53 IS A MOLECULAR SWITCH THAT CONTROLS CANCER CELL FATE DECISIONS DURING OVARIAN CANCER TREATMENT

Cheng, Shuofei¹,³, Julie Lafontaine¹, Hubert Fleury¹,³, Guillaume Cardin¹, Anne-Marie Mes-Masson¹,³, Francis Rodier¹,²,³

¹CRCHUM, Institut du Cancer de Montréal, Montréal, Canada, Université de Montréal, ²Département de Radiologie, Radio-oncologie et médecine nucléaire, ³Programs de biologie moléculaire

Introduction: TP53 is a tumour suppressor gene mutated in approximately 50% of all cancer cases, a proportion also found in ovarian clear cell carcinomas (OCCC). In response to DNA damage, the protein encoded by TP53 (p53) is part of complex signaling cascades (switches), which control immediate cell fate decisions including transient cell cycle arrest, permanent growth arrest (senescence) and cell death. Our hypothesis is that p53 has an impact on cancer cell fate decisions in response to platinum/taxane-based drugs, which trigger massive DNA damage.

Methods: To assess this question we induce DNA damage in OCCC cells using irradiation or platinum/taxane-based chemotherapy in the presence of absence of endogenous p53. Stable depletion of p53 is achieved via the infection of cancer cells with a lentivirus containing a short hairpin RNA targeting TP53 (shp53) and/or by expressing a p53 genetic suppressor element (GSE). We determine treated cell fates by monitoring cell cycle arrest, various cell deaths, and cell senescence using FACS and immunofluorescence.

Results: We observe that p53 ablation in OCCC cells shift immediate cell fate decisions away from senescence and increases long-term cell survival (colony formation). Unlike p53 wild-type cells, p53-manipulated cells continue to divide in the presence of DNA damage leading to aberrant mitosis, the formation of polyploid cells, and mitotic catastrophe (death). While the loss of p53 immediately redirects cells to death after treatment, p53 deficient cells that survive mitotic catastrophe are more proliferative compared to p53 wild-type cells undergoing senescence.

Conclusion: Overall our results suggest that p53-mediated senescence is beneficial for tumour suppression following treatment in OCCC cells and strategies to enhance this effect could be explored.

Outcome/Impact: We propose that the manipulation of cell fates using molecular switches like p53 to favor senescence could benefit long-term survival of selected cancer patients by reducing treatment resistance. In a context of personalized medicine, a strategy using small molecule p53 activators could be useful for patients with p53 wild-type cancer.
78. VALIDATION OF THERAPEUTIC TARGETS IN OVARIAN CANCER

Communal, Laudine¹, Mauricio Medrano²,³,⁴, Fabrice Sircoulomb³,⁴, Isabelle Clément¹, Diane Provencher⁵,⁶, Robert Rottapel²,³,⁴,⁵,⁷, Anne-Marie Mes-Masson¹,⁶,⁸

¹Institut du cancer de Montréal / CRCHUM, Montreal, QC; ²Department of Medical Biophysics, University of Toronto; ³OICR and TFRI; ⁴Princess Margaret Cancer Center and University Health Network, Toronto, ON; ⁵Campbell Family Cancer Research Institute, Toronto, ON; ⁶Department of Obstetrics and Gynaecology, Université de Montréal; ⁷Samuel Lunenfeld Research Institute, Toronto, ON, ⁸Department of Medicine, Université de Montréal

Introduction: The TFRI/OICR Selective Therapy Program (STP) has identified new promising candidates to improve cancer outcomes using an integrative genomic, proteomic and functional approach (1). Here we characterized and validated the relevance of selected candidates as therapeutic targets or biomarkers in High Grade Serous Epithelial Ovarian Cancer (HGS-EOC).

Methods: A systematic candidate characterization included expression evaluation by Western blot in numerous EOC cell lines. Candidate expression was evaluated by immunofluorescence (IF) on a tissue-microarray (TMA) consisting of 101 cases of HGS-EOC. Multi-labeling IF conditions were defined to allow the discrimination of epithelial and stromal cells as well as nuclei and cytoplasmic compartments. Candidate expression was accurately quantified in each specific compartment with an image analysis procedure. Expression levels were correlated with patient clinical parameters in order to determine their relevance and to prioritize them for further studies.

Results: Analysis was completed for 12 candidates. We obtained promising results for four candidates. Among them, high expression of CD151 protein was correlated with poor patient prognosis in our HGS-EOC TMA and was therefore subjected to analysis in the pan-Canadian TFRI COEUR TMA cohort comprised of 833 HGS-EOC cases. Low JAMA and CD58 expression was correlated to a poor prognosis. We also observed that high stromal to epithelial expression of APBB3 was associated with poor prognosis.

Conclusions: All the candidates showed good expression in HGS-EOC suggesting that they can be further studied as therapeutic targets. Only APBB3 showed higher expression in the stromal compartment of tumours, although this was only seen in a subset of patients. In addition, some of the STP markers can be further investigated as prognostic markers in HGS-EOC.

Outcome/Impact: Systematic review of all STP candidates will reveal those best suited to be further studied as therapeutic targets or prognostic markers.

79. IN VIVO GENOMIC SCREENING IDENTIFIES NOVEL EFFECTORS OF RETINOIC ACID THERAPY IN TRIPLE-NEGATIVE BREAST CANCER

Coyle, Krysta¹, Shelby Clattenburg², Rong-Zong Liu³, Cheryl Dean¹,², Mohammad Sultan¹, Carman Giacomantonio¹,⁴, Lucy Helyer⁴, John Mackey³, Roseline Godbout³, Paola Marcato¹

Departments of ¹Pathology, ²Microbiology & Immunology, and ⁴Surgery, Dalhousie University, Halifax NS; ³Department of Oncology, University of Alberta, Edmonton AB.

Introduction: Triple-negative breast cancers (TNBCs) can be defined by molecular subtypes with differing outcomes and response to therapeutics. Recent data suggests that retinoic acid (RA) has opposing effects in TNBCs, depending on the molecular subtype: inhibiting in basal-like MDA-MB-468 and promoting in mesenchymal-like MDA-MB-231. Identifying the genes responsible for these effects will reveal the mechanism of a pathway affecting the rate of TNBC progression.

Method: We performed in vivo, unbiased, total-genome knockdown screens in MDA-MB-468 and MDA-MB-231 cells to identify genes that functionally contribute to the opposing effects of RA. Identified genes were validated by qPCR expression analyses, in vitro proliferation assays, and tumour growth assays. Patient tumour gene expression and survival data were compiled for selected genes.

Results: 28 genes were identified as potential contributors to RA’s tumour-inhibiting effects in basal-like TNBCs. The expression of five of these genes is affected by RA or the RA-producing enzyme ALDH1A3. We have identified at least three genes as potential novel mediators of RA’s tumour inhibiting effects, and will verify these in tumour xenograft experiments. 50 genes were identified as probable effectors of RA’s tumour-promoting effects in mesenchymal-like TNBCs and subjected to further characterization. The expression of 18 of these genes is affected by RA. We have demonstrated that two of these genes, voltage-gated sodium channel 1α (SCN1A) and gamma-aminobutyric acid receptor α3 (GABRA3), contribute to tumour growth, metastasis and patient survival.

Conclusion: We have identified several novel effectors of RA signaling (e.g. SCN1A, GABRA3). Functional characterization of these genes will allow us to determine the mechanisms by which RA induces opposing growth effects in breast cancer.

Outcome/Impact: An understanding of the context-specific mechanisms governing the effects of RA is required to achieve clinical success. Results from this study will increase the efficacy of RA as a cancer therapeutic and improve TNBC patient outcomes.
80. FLCN, A NOVEL NEGATIVE REGULATOR OF AMPK-DEPENDENT CANCER METABOLIC ADAPTATION

Gingras, Marie-Claude\textsuperscript{1,2}, Ming Yan\textsuperscript{1,2}, Zahra Jalali\textsuperscript{1,2}, Elite Possik\textsuperscript{1,2}, and Arnim Pause\textsuperscript{1,2}

\textsuperscript{1}Goodman Cancer Research Center, McGill University, Montréal, Québec, H3A 1A3, Canada; \textsuperscript{2}Department of Biochemistry, McGill University, Montréal, Québec, H3G 1Y6, Canada.

Introduction: Altered cellular metabolic reprogramming is a common hallmark of cancer, particularly essential during cancer progression. To fulfill the energetic demand required to drive aberrant cell proliferation and overcome metabolic pressure induced by environmental stress, such as nutrient deprivation, cancer cells commonly adapt their metabolism by hijacking metabolic adaptation programs. AMP-dependent kinase (AMPK) is the master regulator of energy homeostasis but its role in cancer is controversial as both AMPK pro- and anti-tumourigenic functions were reported. Germline mutations in the gene encoding the tumour suppressor folliculin (FLCN) lead to Birt-Hogg-Dubé (BHD) syndrome, which is associated with an increased cancer risk. We recently identified FLCN, an AMPK binding partner, as a novel negative regulator of AMPK-dependent cancer metabolic reprogramming.

Methods: Using mouse embryonic fibroblasts (MEFs) and cancer cell lines from various origins depleted for FLCN expression, we evaluated FLCN roles with respect to AMPK-dependent energy functions.

Results: Strikingly, we demonstrated that loss of FLCN constitutively activates AMPK, resulting in cellular metabolic reprogramming characterized by increased cellular oxidative phosphorylation and aerobic glycolysis capacity. This metabolic change boosts cellular energy levels and biosynthetic precursor production and confers cellular resistance to metabolic stress as well as tumourigenic advantage in vitro and in vivo.

Conclusions: We identified FLCN as a physiological negative regulator of AMPK. Importantly, our data suggest that beyond its direct role in BHD disease, FLCN might be commonly involved in metabolic adaptation process occurring during cancer progression.

Outcome/Impact: Our findings identify FLCN as a new negative regulator of AMPK and shed light on the tumour suppressor mechanism of FLCN. Moreover, our data strongly suggest that by controlling AMPK-dependent cancer metabolic adaptation, FLCN is a novel major player that control metabolic adaptation and metabolic stress resistance during cancer progression process.
81. NOVEL THERAPEUTIC TARGETS IN HEAD AND NECK CANCER

Kondratyev, Maria¹, Troy Ketela², Azin Sayad², Stephano Marastoni¹, Carl Virtanen³, Laurie Ailles³, Alessandro Datti⁵, Mikhail Bashkurov³, Jason Moffat², Reider Grenman⁴, Marianne Koritzinsky¹, Bradly G. Wouters¹

¹Princess Margaret Cancer Centre, Department of Radiation Oncology, University Health Network, Toronto; ²Banting & Best Department of Medical Research; ³Ontario Institute for Cancer Research; ⁴Dept. of Otorhinolaryngology - Head and Neck Surgery, Turku University and Turku University Hospital, Turku Finland, ⁵Samuel Lunenfeld Research Institute – Mount Sinai Hospital

Introduction: Head and neck carcinoma is the sixth most common malignancy in the world. Despite advances in diagnosis and treatment, the survival rates remain unacceptably low due to high rates of local and regional recurrences. The biology of underlying patients with poor prognosis remains unclear.

Methods: We used functional genomic technologies to identify new potential therapeutic targets for metastatic disease in HNSCC. These targets were identified by conducting whole genome shRNA screens in matched sets of cell lines derived from primary tumours and their respective metastatic sites, with the goal of identifying genes that become essential for cell survival only following metastasis. To test if knockdown of selected targets will inhibit metastasis in a therapeutic setting, we established orthotropic model of HNSCC that metastasize to regional lymph nodes in the mouse.

Results: Several components of the Notch signaling pathway (Notch3, Jag2) were identified and validated as essential for survival of cells derived from metastatic sites. In one patient, whole exome sequencing identified a novel mutation in one of the EGF domains of Notch3 that was acquired only in the metastatic line. Mutations in EGF domains have been reported to influence interaction with specific ligands, dictating which ligand can activate Notch signalling. Our preliminary data indicate that metastatic, but not primary tumour cells, undergo apoptosis upon knockdown of Notch3 and that a distinct set of target genes is induced upon interaction between Notch3 and the Jag2 ligand (expressed at considerably higher levels in metastatic cells). Furthermore, our results to date indicate that suppression of Notch3 improves survival in mice bearing orthotrophic tumours derived from the metastatic HNSCC lines.

Conclusions: Our data demonstrate that metastatic cells from head and neck tumours acquire dependency on Notch3 signaling. Novel treatments targeting components of this pathway may prove effective in targeting metastatic cells alone or in combination with conventional therapies.
82. PIWI-INTERACTING RNA TRANSCRIPTOME ANALYSES IDENTIFY CANCER TYPE-SPECIFIC EXPRESSION PATTERNS AND SIGNATURES PREDICTING LUNG CANCER PATIENT OUTCOME

Martinez, Victor D.¹, Katey S.S. Enfield¹, Emily A. Vucic¹, Stephen Lam¹, Wan L. Lam¹

¹Integrative Oncology, British Columbia Cancer Research Centre, Vancouver, BC, Canada

Introduction: PIWI-interacting RNAs (piRNAs) are short RNA species known to play a key role in the epigenetic regulation of germ cell genomes; and their role in somatic tissues and cancer is emerging. We characterize piRNA transcriptomes from malignant and non-malignant tissues for the purpose of identifying novel targets for prognostics and therapy.

Methods: We deduce expression levels from 20,831 piRNAs in 6,260 human piRNA transcriptomes from 11 organs by developing a custom small RNA sequencing pipeline. Association of individual piRNAs with survival was evaluated using the log-rank method. Multiple-piRNA survival signatures were identified using Cox Proportional Hazard models. Patient “risk scores” were calculated by multiplying expression values of each piRNA in the model by their respective Cox coefficient, and then summing their values. High- and low-risk tertiles were compared by Kaplan-Meier survival analysis.

Results: Our results were surprising: 1) only 1-3% of known piRNAs are expressed in somatic tissues (compared to germline cells), 2) somatically-expressed piRNA genes are mainly derived outside germline “piRNA clusters”, 3) piRNAs expression can distinguish tissue-of-origin, suggesting tissue-specific functions, 4) tumours differ from non-malignant tissues in cancer-type specific expression pattern, 5) piRNA expression delineates clinical features relevant to individual cancer types, and 6) specific expression patterns distinguish adenocarcinoma and squamous cell carcinoma sub-types of lung cancer, and provide prognostic signatures.

Conclusions: We have identified somatically-transcribed piRNA loci in human non-malignant and malignant tissues. Our findings provide clear evidence of somatic, tissue-specific expression of piRNAs which we have made available as a public resource for the prioritization of piRNAs for future studies. We show that their malignant expression patterns contribute to cancer type-specific biology. Furthermore, we have identified piRNA signatures that have prognostic value for the specific subtypes of lung cancer.

Outcome/Impact: This is the first study to comprehensively analyze the expression of piRNAs in non-malignant and malignant tissues. Our findings suggest that piRNAs have potential utility for prognostics, and their cancer-type specific expression patterns can be explored to better understand the unique biology of different cancers and their subtypes.
83. A NANOPARTICLE-BASED STRATEGY TO CIRCUMVENT THE “GOLDILOCKS EFFECT” IN PIGMENT-ENHANCED PHOTOTHERMAL THERAPY

Ng, Kenneth¹, Weersink Robert¹,³, Wilson Brian C.¹,², Zheng Gang¹,²

¹Princess Margaret Cancer Centre and Techna, UHN, Toronto; Departments of ²Medical Biophysics and ³Radiation Oncology, University of Toronto, Toronto

Introduction: Pigment-enhanced photothermal therapy is a form of laser-induced thermal therapy that utilizes an exogenously-delivered, light-absorbing agent to convert laser energy into heat. Co-localization of the pigment and light results in thermal ablation of the tumour tissue through the light-heat conversion capabilities of the pigment. The prevailing theory is that by maximizing absorption in the target tissue, a greater therapeutic effect can be achieved. Nanoparticle-based photothermal agents have been developed with this idea in mind. However, one overlooked problem is the fact that increased light absorption also results in increased light attenuation at depth in tissue. Hence, a “Goldilocks effect” arises; how does one achieve the optimal tissue absorption without going too high (reduced light penetration) or too low (inadequate heating)? Herein, we examine a nanoparticle-based strategy to circumvent this challenge.

Method: A photothermal agent with self-regulating capabilities was made by incorporating molecular aggregates within a temperature-responsive nanovesicle. Increasing the temperature above the lipid phase transition (T_m=41°C) results in reduced light absorption. These molecular aggregates were embedded within a hydrogel phantom and irradiated with a high-power laser source at the wavelength of the nanoparticle’s absorption peak (824 nm). A non-temperature-responsive agent was used as a control. Light transmission and thermographic data were collected.

Results: Irradiation of the photothermal agent led to a rise in temperature of the phantom over time. We observed non-linearity in light transmission for the temperature-responsive agent, which was not observed in the control. Furthermore, temperature changes measured by infrared thermography showed the irradiated area in the temperature-responsive sample maintained a uniform temperature (~41°C), while the control displayed heterogeneous heat distribution resulting from excess light absorption at the gel surface.

Conclusion: In summary, we designed a nanoparticle-based photothermal agent that exhibits thermo-responsive behavior. We have shown in phantoms that these nanomaterials allow for uniform heating of the irradiated volume at depth when compared to controls. Future research will focus on computational modelling of thermal/light diffusion in both systems and additional studies within tissue-mimicking phantoms and in vivo solid tumours.

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84. EARLY DETECTION OF ULTIMATE RESPONSE TO CHEMOTHERAPY IN BREAST CANCER PATIENTS USING A MULTI-MODAL IMAGING STRATEGY

Sadeghi-Naini, Ali$^{1,2}$, William Tran$^1$, Vivian Yau$^1$, and Gregory Czarnota$^{1,2}$

$^1$Department of Radiation Oncology and Physical Science, Sunnybrook Health Sciences Centre; $^2$Departments of Medical Biophysics and Radiation Oncology, University of Toronto

Introduction: Breast cancer patients respond heterogeneously to chemotherapy. As such, a predefined regimen is not often effective for all patients. This makes an early detection of patients who are refractory to a specific therapy critical, since this could facilitate a change to a more effective treatment. The efficacy of three functional imaging techniques was investigated in this study for an early evaluation of response to chemotherapy in breast cancer patients.

Methods: Locally advanced breast cancer patients receiving neoadjuvant chemotherapy were monitored during their course of treatment using quantitative ultrasound (QUS) techniques for detecting tumour cell death, ultrasound elastography for measuring changes in tumour stiffness, and diffuse optical spectroscopy (DOS) to evaluate alterations in tissue metabolism, all in response to treatment. Ultimate responses of patients to chemotherapy were determined clinically and pathologically. Several mean-value and textural biomarkers were extracted from the images and evaluated for an early prediction of treatment response.

Results: Obtained results demonstrated statistically significant differences in changes measured in textural biomarkers extracted from QUS and DOS parametric images between treatment responders and non-responders, one and four weeks after the start of treatment. Changes in relative tumour stiffness demonstrated statistically significant differences only after four weeks of treatment. Results of discriminant analyses suggested that the treatment responding and non-responding patients can be differentiated after one and four weeks of treatment with high sensitivity and specificity (80-100%) using the proposed imaging biomarkers in a hybrid profile.

Conclusions: This study suggests for the first time that QUS imaging, ultrasound elastography, and DOS in conjunction with textural analysis techniques can be used non-invasively to predict response of breast cancer patients to chemotherapy within days after treatment initiation.

Outcome/Impact: This work, thus, establishes a framework for an early detection of chemotherapy-refractory patients using non-invasive image-based hybrid biomarkers of treatment response. Such early insight could potentially facilitate switching an inefficient treatment regimen to a more effective one on an individual patient basis, resulting in an increased number of complete pathologic responses, higher survival rates, and better quality of lives for cancer patients.
85. C4 THERAPEUTIC AXIS – OMICS ANALYSIS OF SEQUENTIAL METASTATIC BIOPSIES IN COLORECTAL CANCER PATIENTS REVEALS GENOMIC ALTERATIONS THAT EVOLVE DURING FIRST-LINE THERAPY

Couëtoux du Tertre, Mathilde¹, Ryan Morin², Suzan McNamara³, Rosemary McCloskey⁵, Rebecca Johnston³, Daniel Fornika², Spring Holter⁴, Steven Gallinger⁴, Gerald Batist⁵

¹QCROC, Montreal, QC; ²SFU, Vancouver BC; ³UBC, Vancouver, BC; ⁴Mount Sinai Hospital, Toronto, On; ⁵Jewish General Hospital, Montreal, QC

Introduction: The Canadian Colorectal Cancer Consortium (C4) was initiated in 2012 with the overall objective of establishing a molecular-based approach to translational cancer care that will improve the outcome of colorectal cancer (CRC) patients. Two research axes were integrated with the primary objectives of accurately stratifying patients with incident CRC according to familial risk (Screening Axis) and by identifying biomarkers that will predict drug resistance (Therapeutic Axis). The Therapeutic Axis is building on the efforts of the Q-CROC-01 study established by the Quebec Clinical Research Organization in Cancer (Q-CROC).

Methods: The main objective of the Therapeutic Axis is to identify biomarkers that can predict molecular signatures linked to therapeutic resistance. This study is ongoing and approved at 14 Canadian sites with 104 patients enrolled so far. Patients with metastatic CRC receiving FOLFOX and bevacizumab consented to 3 tumour biopsies at pre-treatment and at resistance. Twenty-five patients were profiled using WES (tumour and germ line), RNAseq, low pass WGS and miRNA analysis. Serial bloods were also collected for analysis.

Results: Our results detected depletion and enrichment in variant allele fractions of somatic mutations over the course of treatment, the latter of which may indicate subclonal and acquired “driver” mutations that confer therapeutic resistance. A number of genes show recurrent evidence for changes in clonal enrichment at the time of relapse across several patients. Plasma-derived ctDNA will be analyzed to detect the patient’s mutational status during their course of treatment. The extent of genomic heterogeneity in metastatic CRC by analysis of multiple intra-patient post-biopsies will also be explored.

Conclusions: Our study reveals molecular changes over time, or resulting from the selection pressure of treatment which provide insights into tumour evolution during first-line chemotherapy of metastatic CRC.

Outcome/Impact: These findings that may hold clues to optimize current therapeutic decision making which may identify potential target pathways for second-line stratification of patients.
86. USING HEALTH ECONOMICS TO INFORM RESEARCH PRIORITIES: EXAMPLES FROM THE PAN-CANADIAN EARLY DETECTION OF LUNG CANCER STUDY

Cressman, Sonya*1,2, Stuart Peacock1,2,3 and the investigators of the pan-Canadian Early Detection Study4

1Canadian Centre for Applied Research in Cancer Control; 2British Columbia Cancer Agency; 3University of British Columbia; 4Various institutions, *Presenting author: scressman@bccrc.ca

Introduction: Screening with low dose computed tomography has been shown to reduce deaths from lung cancer and national guidelines now recommend screening informed individuals who may be at risk of developing the disease. Due to the high incidence of disease and massive amounts of potential screening participants, the costs associated with screening the general population for lung cancer could have major budgetary impacts in Canada.

Method: The TFRI-funded pan-Canadian Early Detection of Lung Cancer study was launched in 2008 with the intent of proving economic evidence of a lung cancer screening program that is affordable within the Canadian health care system. The study used selection criteria prior to screening that stratified potential participants by their individual risk of developing lung cancer, in addition to age and smoking history. Using patient-level cost and outcomes data from the study and control arm data from a major randomized study in the US, we made a Markov cost-effectiveness model to compare risk-stratified lung cancer screening with standard care.

Results: Presented herein are the early results with preliminary inputs from 2537 participants screened in the study. The results are presented in a way that conveys an introduction to the economic methods that can be used to estimate value from stratification approaches in cancer control, such as selection criteria for screening or the effect of differential screening strategies based individual malignancy risk. Results are discussed in the context of other TFRI-funded approaches to cancer control including biomarker and genomics-based risk stratification.

Conclusions: Using cost-effectiveness modelling and evidence from the pan-Canadian study, we characterize the key parameters of lung cancer screening that are critical towards a cost-effective lung cancer screening program in Canada.

Outcome/Impact: Using the example of risk stratification in the Pan-Canadian early lung cancer detection study we show how health economics applied during pre-clinical discovery can lead to more informed decisions about future research priorities.

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87. CANADIAN COLORECTAL CANCER CONSORTIUM – SCREENING AXIS

Holter, Spring1, Aaron Pollett1, Tae Hart1, Paul Karanicolas2, Eugene Hsieh2, Patrick Parfrey3, William Foulkes4, Carl Brown5, David Schaeffer5, Haili Wang6, Jean Deschenes6, Nancy Baxter7, Cathy Streutker7, Suzan McNamara8, Gerald Batist8, Steven Gallinger1, Robert Gryfe1

1Mount Sinai Hospital, Toronto; 2Sunnybrook Health Sciences Centre, Toronto; 3Memorial University of Newfoundland, St. John’s, NL; 4McGill University Health Centre, Montreal; 5Providence Health Care, Vancouver; 6Alberta Health Services, Edmonton; 7St. Michael’s Hospital, Toronto; 8Consortium de Recherche en Oncologie Clinique du Québec, Montreal

Introduction: The overall objective of the Canadian Colorectal Cancer Consortium (C4) is to establish a molecular-based approach to translational cancer care that will improve the outcome of colorectal cancer patients through two integrated aims. The Screening Axis will improve the impact of early diagnosis and decrease the cost of managing colorectal cancer (CRC) through targeted screening of families stratified by risk. The Therapeutic Axis will improve the life expectancy and reduce the cost of management of advanced CRC through the study of drug resistant metastatic disease and the development of biomarker gene panels to predict drug resistance.

Methods: The Screening Axis has four goals: 1) Activate clinical sites for recruitment of incident patients under age 60 with colorectal cancer; 2) Recruitment of patients, collection of biospecimens, performance and review of DNA mismatch repair immunohistochemistry (IHC), and pedigree risk classification; 3) Recruitment of relatives and dissemination of screening information to family members; 4) Create a unique and unprecedented biobank from high-risk families for germline gene discovery efforts.

Results: Seven sites in five provinces have been initiated and are actively recruiting patients under age 60 with CRC and 45% (224/500) of proband recruitment is complete. Mismatch repair IHC has been performed on 195 of the 224 participants recruited. Twenty-three (12%) have been IHC deficient and 52% (12/23) have had a germline mismatch repair mutation identified. First-degree relatives of recruited probands are also eligible for recruitment and 14.5% (106/729) of eligible family members have been consented. Blood samples have been donated by 172 participants and fresh tumour and normal pairs have been collected on 31 participants.

Conclusions: Proband recruitment has been successful in the first two years of the C4 and we expect to meet our goal of 500 proband participants. Performance of mismatch repair IHC has been effective with the expected IHC deficiency and germline mutation rate. Kin recruitment has been lower than expected; however, we anticipate increased kin participation as proband recruitment winds down.

Outcome/Impact: This is the first multi-site pan-Canadian high-risk colorectal cancer registry established in Canada. We encourage that a similar model be used for future collaborations.
88. IDENTIFICATION OF ONCOGENIC NETWORKS THAT CO-OPERATE WITH RB-LOSS TO INDUCE METASTATIC BREAST CANCER

Jiang, Zhe ¹, Li Huiqin ¹, Skowron Patryk ², Garzia Livia ², Adam Jes ², Egan Sean ², Dupuy Adam ³, Taylor Michael ², Zacksenhaus Eldad ¹

¹Toronto General Research Institute; ²The Hospital for Sick Children; ³University of Iowa

Introduction: We previously showed that deletion of murine Rb in mammary epithelium induces divergent mammary tumours that clustered primarily with triple negative (TNBC) or luminal B tumours. The former tumours contained p53 mutations, and accordingly, deletion of both Rb and p53 induced TNBC-like tumours (Jiang et al, J. Clinical Investigation, 2010; Jones et al., submitted). To identify oncogenic pathways that co-operate with RB-loss to promote metastatic breast cancer, we performed a sleeping beauty (SB) transposon mediated mutagenesis screen in vivo.

Methods: Two cohorts of compounded mice carrying the SB transposase, the transposons (integrated into 2 different chromosomes), floxed Rb allele, and MMTV-Cre were generated. 120 primary mammary tumours and 80 lung metastases with confirmed Rb-deletion, as well as 40 control mammary tumours (without Rb-deletion) were subject to ligation-mediated PCR, deep-sequencing and bioinformatic analysis to identify gene-centric common integration sites (gCIS).

Results: We identified (1) 74 gCISs in primary tumours, 48 gCISs in lung mets and 9 in control tumours; (2) 8 gCISs both in mammary primary and lung metastases (3 of which were also found in control). Targeting these 8 Shared Maintenance Genes may block growth/progression of both primary and metastatic tumour cells; (3) 40 gCISs exclusively in mets; (4) several gCISs with distinct integrations sites in different mets of the same mouse (convergence analysis), suggesting that they are preferentially targeted and therefore presumably important for metastasis in combination with Rb-loss. Some of these gCISs represent common pathways and/or are known to be lost/activated in human breast cancer.

Conclusions: RB loss co-operates with several unique genes/pathways to induce metastatic breast cancer. Functional analysis of some of these gCISs is underway.

Outcome/Impact: Although RB loss is not druggable, its cooperating oncogenic pathways, identified here by SB mutagenesis, may be amenable to therapeutic intervention.

This research is funded by the Terry Fox Foundation.
89. COEUR: A PAN-CANADIAN PLATFORM FOR THE DEVELOPMENT OF BIOMARKER-DRIVEN SUBTYPE SPECIFIC MANAGEMENT OF OVARIAN CARCINOMA


Introduction: Ovarian cancer is a leading cause of cancer-related deaths in the Western world due to a high rate of resistance to standard first-line chemotherapies. In 2009, through Terry Fox Research Institute, leading ovarian cancer researchers and clinicians across Canada joined forces to develop a biomarker-driven subtype-specific management of ovarian carcinoma. Biomarkers have the potential to enhance diagnostic reproducibility of histological type as well as guide therapy as predictive markers. In order to bring biomarker research to the next level, the COEUR program proposes to develop a platform to validate biomarkers that can be used in the stratification of ovarian cancer patients that results in an improved clinical management.

Methods: An essential component of the project is the creation of a Canadian Ovarian Experimental Unified Resource (COEUR), a repository of biospecimens and associated clinical data with control quality assessment. For this purpose COEUR has collected formalin-fixed paraffin embedded (FFPE) tissues, frozen tissues, serum, plasma, ascites fluids anduffy coat for DNA extraction. Quality assessment (QA) of FFPE samples is done on each case by standard pathological review and biomarker staining. QA of DNAs was determined by nested PCR amplification of the beta-globin gene. Biospecimens are openly shared. Investigators who want to gain access to the platform have to complete an application form which is subject to review by the study committee. The COEUR application is available here: http://www.tfri.ca/en/research/translational-research/coeur.aspx.

Results: COEUR has collected 2000 cases. FFPE blocks were used to build TMA for high-grade serous (n=750), endometrioid (n=200), clear cells (n=200) and mucinous subtypes (n=50). This collection counts also more than 800 blood DNAs and 250 tissue DNAs with 80% being of high quality. Clinical data are regularly reviewed and updated. More than 10 studies have been granted access to the cohort for biomarker validation.

Conclusions: This central platform establishes biological and data resources for the research community, and will help serve the entire biomarker program COEUR and to facilitate biomarker research by promoting the translation of new findings into the clinical arena.

90. IDENTIFICATION OF BIOLOGICALLY AGGRESSIVE PROSTATE CANCER CASES IN THE LOWEST RISK CATEGORY OF PATIENTS ENROLLED IN THE QUEBEC PROCURE BIOBANK: A SMALL BUT CRITICAL GROUP FOR IN-DEPTH MOLECULAR CHARACTERIZATION

McKercher, G.1, Saad F2, Lacombe L3, Carmel M4, Mes-Masson A-M2, Bergeron A3, Piché A4, Brimo F5, Latour M2, Têtu B5, Doueik A4, Chevalier S1,5, Aprikian AA1,5

1PROCURE Alliance, and 2Montréal, 3Québec, 4Sherbrooke and 5McGill University Health Centres, Québec, Canada.

Introduction: Prostate cancer (PCa) ranks first among malignancies in Canada and 3rd for death by cancer. It is believed that low-risk (LR) cancers, as identified mainly by PSA and histologic grade, do not pose a significant threat and as such are offered active surveillance. The aim of this study was to identify within the PROCURE Biobank LR cases that demonstrated early recurrence, in order to form a cohort for in-depth molecular characterization and identification of potential biomarkers of prognosis.

Methods: The PROCURE Biobank comprises 2020 patients enrolled between 2007 and 2012 in four university hospitals: Montreal (CHUM, MUHC), Quebec (CHU de Québec) and Sherbrooke (CHUS). From these, 97% (n=1969) underwent prostatectomy, biobanking, and serial prospective biosample recollection. We searched for cases with Gleason score (GS) 6 (pure grade 3 cancer), PSA ≤ 10 ng/ml, pT2, and negative surgical margins, that demonstrated PSA recurrence within 3 years from surgery.

Results: The cohort characteristics were: average age 62±6 years old, PSA 8.0±10.7 ug/L, surgery GS 6 (23%), 7 (66%), ≥ 8 (11%), stages pT2 (62%), pT3a (27%), pT3b (11%); 24% PSA recurrence after a 3 year-median follow up, with overall and cancer-specific death rates of 3% (n=58) and 0.6% (n=11), respectively. We identified 314 LR cases of pure GS 6, of which 238 had pT2 and negative margins and a median follow-up of 42 months. Among these, 11 cases (4.6%) demonstrated early biochemical recurrence at a median of 27 months.

Conclusions: We have identified a subset of potentially aggressive LR cancers of pure GS 6, no extraprostatic extension (focal or established) and negative surgical margins. The variety of fresh biosamples available from these cases could serve for intense molecular and genetic characterization in the hopes of identifying biomarkers of prognosis.

“The data used for this research were obtained from the PROCURE Biobank / Cancer Research Society Partnership”.
91. THE CANADIAN PROSTATE CANCER BIOMARKER NETWORK (CPCBN): A VALIDATION COHORT FOR PROSTATE CANCER BIOMARKERS

Ouellet, Véronique¹, Véronique Barrès¹, Nathalie Delvoye¹ N, Mathieu Latour¹, Dominique Trudel¹, Armen Aprikian², Alain Bergeron³ A, Barbara Bowes⁴, Fadi Brimo², Robert Bristow⁴, Karen Chadwick⁴, Simone Chevalier², Darrel Drachenberg⁵, Ladan Fazli⁶, Neil Fleshner⁴, Martin Gleave⁶, Tara Horrill⁵, Hélène Hovington³, Pierre Karakiewicz¹, Jeffrey Klotz⁷, Laurence Klotz⁷, Louis Lacombe³, Anne-Marie Mes-Masson¹, Alireza Moeen⁶, Eleonora Scarlata², Paula Sitarik⁵, Jenna Sykes⁴, Theodorus van der Kwast⁴, and Fred Saad¹.

¹CHUM/CRCHUM; ²MUHC/RC-MUHM; ³CHU de Québec; ⁴UHN; ⁵MPC; ⁶VPC; ⁷Sunnybrook;

Introduction: The CPCBN is a program that gathers researchers from several institutions from four different Canadian provinces. The main objective of this program is to address important issues dealing with prostate cancer diagnosis and management.

Methods: The CPCBN created a validation cohort composed of 1508 patients treated by radical prostatectomy (RP). Normal and tumoural tissues from RP specimens were assembled on tissue microarrays (TMAs). Two additional cohorts of 125 intermediate risk patients and 250 patients followed by active surveillance, for whom biopsy specimens are available, are currently being completed. Diagnosis, treatment and clinical outcome data have been collected in database for all these patients. Researchers who want to validate prognostic biomarkers can access the CPCBN cohort, in a step-wise manner, upon presentation of supporting preliminary data (tfri.cpcbn@gmail.com).

Results: The mean age at diagnosis of the 1508 patients composing the CPCBN RP cohort is 61.4 years. Of these patients, 31% had low grade (≤6), 55% had grade 7 and 14% had high grade (≥8) prostate cancer. Biochemical relapse occurred in 30% of the patients and 3% developed bone metastasis. The median follow-up of the cohort is 87.5 months. TMAs composed of 2 adjacent benign and 3 tumour tissue cores for each patient have been completed. Quality control of TMAs has been performed by a single pathologist who requested correction whenever necessary. To date, the program has accepted proposal for the validation of six biomarkers (p65, AR, CCN3, p27, Ki67, NKX3.1/MYC).

Conclusions: The CPCBN RP cohort is representative of the prostate cancer disease seen in the Canadian population. The number of biochemical relapses and bone metastasis events in the cohort is large enough to allow evaluation of prognostic biomarkers with sufficient power.

Outcome/Impact: Meta-analysis of multiple markers can ultimately define a nomogram of combined biomarkers for early treatment decisions in men newly diagnosed with prostate cancer.
92. CAPTURING MEN’S PERSPECTIVES ABOUT ACTIVE SURVEILLANCE
CANADIAN PROSTATE CANCER BIOMARKER NETWORK

Fitch, Margaret¹, Kittie Pang¹, Veronique Ouellet², Simone Chevalier³, Darrel E. Drachenberg⁴, Antonio Finelli⁵, Lucie Hame²³, Kathy Li⁵, Jean-Benoit Paradis⁶, Maureen Palmer⁷, Paula Sitarik⁴, Simon Sutcliffe³⁸, Alan So⁷, Simon Tanguay³, Fred Saad², and Anne-Marie Mes-Masson²

¹Sunnybrook Research Institute; ²Centre Hospitalier de l’Université de Montréal and CRCHUM; ³McGill University Health Centre; ⁴Manitoba Prostate Center; ⁵UHN; ⁶Centre de Santé et des Services Sociaux de Chicoutimi, ⁷Vancouver Prostate Center; ⁸TFRI, Vancouver

Introduction: Men with low risk prostate cancer are being offered an active surveillance (AS) approach rather than undergoing curative surgical or radiation treatment. This approach runs contrary to the traditional message of undergoing treatment as soon as possible following a cancer diagnosis. A deeper understanding of men’s perspectives on AS will help identify factors that influence their decision to undertake AS and assist health professionals in having conversations about this option.

Methods: Focus group interviews (n=7) were held in several Canadian cities with men (N=56) who had been diagnosed with prostate cancer and had been eligible for AS at diagnosis. The men’s viewpoints were captured regarding their understanding of AS, the factors that influenced their decision to engage in AS, and their experience with the approach. A content and theme analysis was performed on the verbatim transcripts from the sessions.

Results: The majority of the focus group participants were on AS at the time of the session. All described the idea that their disease was not “large enough” to require treatment. By postponing curative treatments they could avoid the side effects and continue with their current quality of life. They felt comfortable because of the close monitoring they were undergoing and the idea that they could receive treatment if needed. Although the participants described taking active steps to seek information about their disease, the conversation with the doctor, and how AS was explained, was a key influence in their decision. Other important factors in choosing, or not, AS included lack of information about treatment options, low confidence in the health system, understanding of their own disease state, personal perspectives on quality of life, the experiences of others with prostate cancer, and attitudes expressed by others. Decisions to discontinue AS were linked to disease progression.

Conclusions: AS is a relatively new approach for the care of men with low risk prostate cancer. However, men need to have clear explanations to make informed decisions about engaging in this approach.

Outcome/Impact: Altogether these findings will lead to the development of a detailed questionnaire to interrogate a larger cohort of low risk prostate cancer patients.
93. LOSS OF FLCN CONFEROS OSMOTIC STRESS RESISTANCE VIA AMPK-DEPENDENT REMODELLING OF CARBOHYDRATE STORES

Pause, Arnim\textsuperscript{1,2}, Elite Possik\textsuperscript{1,2}, David Hall\textsuperscript{3}

\textsuperscript{1}Goodman Cancer Research Center, \textsuperscript{2}Department of Biochemistry, McGill University, Montréal, Québec; \textsuperscript{3}Department of Neuroscience, Albert Einstein College of Medicine, New York, USA.

Introduction: When the extracellular osmolarity is higher than the intracellular osmolarity, cells experience hypertonic stress, which promotes water flux out of the cell, causing cellular shrinkage, protein and DNA damage, cell cycle arrest and cell death. Recent evidence demonstrated that hypertonic stress significantly contributes to the initiation and progression of many human diseases including cancer, since both renal and non-renal cells are frequently exposed to hypertonic stresses. Birt-Hogg-Dubé is a renal cancer syndrome that arises in patients with inactivating mutations in the FLCN gene. We have recently shown that FLCN is a negative regulator of AMPK, a heterotrimeric protein complex and master regulator of energy metabolism and stress response (Possik et al., 2014 and Yan et al., 2014). We have also shown that loss of FLCN increases resistance to stresses in nematodes and in mammalian cells.

Methods: To characterize the role of the FLCN/AMPK interaction in osmotic stress resistance, we used a genetic approach that employs the model organism \textit{C. elegans} and mammalian cells including FLCN negative mouse embryonic fibroblasts and human kidney cancer cells.

Results: Here we show that loss of FLCN confers resistance to osmotic stress in nematodes and in mammalian cells, which is highly relevant to the role of FLCN as a renal tumor suppressor. Our results indicate that the chronic AMPK activation conferred by FLCN loss increases glycogen levels in \textit{C. elegans} under basal conditions. We further show that under osmotic stress, glycogen is rapidly degraded and synthesized into organic osmolytes which protects the \textit{flcn} mutant animals from water loss and body shrinkage. Importantly, loss of AMPK, glycogen synthase or glycogen phosphorylase, which are critical enzymes in glycogen metabolism, completely suppressed the increased osmotic stress resistance in \textit{flcn} mutant animals. Moreover, this resistance mechanism is evolutionarily conserved. Our data indicate an accumulation of glycogen in the renal tumours from BHD patients.

Conclusions and Outcome: Cancer cells and specifically renal carcinomas encounter hypertonic microenvironments. Our data indicate that renal tumour cells/tissues upon loss of FLCN, become resistant to osmotic stress via AMPK-dependent accumulation of glycogen and organic osmolytes, resulting in increased proliferation and survival.
94. CANADIAN TISSUE REPOSITORY NETWORK BIOBANK CERTIFICATION PROGRAM

Watson, Peter\textsuperscript{1,2,3}, Sheila O'Donoghue\textsuperscript{1,2}, Rebecca Barnes\textsuperscript{1} on behalf of CTRNet

\textsuperscript{1} Canadian Tissue Repository Network (CTRNet); \textsuperscript{2} Office of Biobank Education and Research (OBER), Department of Pathology and Laboratory Medicine, BCCA and UBC, Vancouver; \textsuperscript{3} Tumour Tissue Repository, Trev and Joyce Deeley Research Centre, BCCA, Victoria, BC

Introduction: Difficulty accessing high-quality biospecimens is recognized as a major roadblock to cancer research. Separating signal from noise in sensitive assays and large data sets also demands biospecimens that have been collected, processed, and stored in a standardized fashion. The current drought in drug development pipelines, and failures to reproduce research findings across the translational research landscape, specifically highlight the hazards inherent in poor quality biospecimens. The Canadian Tissue Repository Network (CTRNet; \url{www.ctrnet.ca}) has developed a Biobank Certification program to address national standards for research biobanking.

Methods: CTRNet’s Certification program is applicable to the full spectrum of biobanking and all researchers handling human biospecimens (i.e. all types of biobanks) and is anchored by an education program that addresses key operational, quality and governance aspects of biobanking based on international best practice standards. CTRNet’s Certification program involves two phases: the first phase, known as Registration, involves one member of a research biobank completing a registration form and the online overview education module; the second phase of the program, referred to as Certification, involves provision of additional education modules customized to biobank type, and assessment of documentation to determine that the biobank has adopted best practice standards.

Status: The need for this Certification program is acknowledged across the health research community, and researchers, research institute leaders, and research funders, recognize its value in addressing the issue of access to high quality biospecimens and reproducibility of research data. As of December 2014, seventy-seven biobanks were registered in the program, twenty-three are in the process of certifying, and eighteen have successfully completed certification.

Conclusion: Increased awareness of how to find and access appropriate biospecimens and the impact of pre-analytical variables on biospecimen quality, are key to the future success of Canadian cancer research. Participation in the CTRNet Biobank Certification program gives researchers the means to improve access to biospecimens through building a national register, and assurance that these biospecimen collections conform to known quality standards. CTRNet has also developed complementary tools and services to support all researchers using biospecimens. For more information on Certification go to \url{http://biobanking.org/brc} or contact us at info@biobanking.org.
95. INTEGRATED GENETIC AND EPIGENETIC ANALYSIS OF MODEL AND PATIENT ACUTE MYELOID LEUKEMIAS

Wilhelm, Brian\textsuperscript{3,4,8}, Frederic Barabe\textsuperscript{1,2}, Magalie Celton\textsuperscript{3,4}, Audrey Forest\textsuperscript{3,4}, Anne Bergeron\textsuperscript{1}, Radia Johnson\textsuperscript{3,4}, Laurine Gil\textsuperscript{1}, Angélique Bellemare-Pelletier\textsuperscript{3,5}, Sonia Cellot\textsuperscript{6}, Josée Hebert\textsuperscript{7,8}, Etienne Gagnon\textsuperscript{3,5,8}

\textsuperscript{1}Centre de Recherche du Centre Hospitalier de l’Université Laval, Quebec City, Canada; \textsuperscript{2}Department of Medicine, Université Laval; \textsuperscript{3}Institute for Research in Immunology and Cancer; \textsuperscript{4}Laboratory for High throughput biology; \textsuperscript{5}Laboratory for Cancer Immunobiology; \textsuperscript{6}Division of Hematology, Ste-Justine Hospital, Montréal, Canada; \textsuperscript{7}Division of Hematology, Maisonneuve-Rosemont Hospital, Montréal, Canada; \textsuperscript{8}Department of Medicine, Université de Montréal, Montréal, Canada.

Introduction: Next generation DNA sequencing has provided significant insights into the genetic determinants of acute myeloid leukemia (AML). Large scale sequencing studies of AML patient cohorts have revealed a remarkable level of genetic heterogeneity between patients who nevertheless have the same disease phenotype.

Methods: As a solution to the problem of extensive genetic diversity between patients, we have modified a previously published model system in order to generate multiple human leukemias from CD34+ cord blood cells from a single healthy donor. A human MLL-AF9 (MA9) fusion gene is retrovirally introduced into the donor cells which are then cultured for 30 days before being transplanted into immunocompromised (NSG) mice that subsequently develop either AML or acute B-cell lymphoblastic leukemia (B-ALL) after ~24 weeks.

Results: We have generated and performed RNA-seq on 22 leukemias from 4 single donors and compared these data to RNA-seq data we have generated for pediatric MA9-AML patients revealing 34 candidate genes with an expression pattern highly specific for MA9 AMLs. We have also examined the DNA methylation changes at each stage in our model system using the Methyl-Seq capture survey approach along with several primary patient samples with MLL translocations. Functional assessment of specific candidate genes through shRNA knock-down experiments has shown that at least some of these candidate genes, which are known oncogenes in other tumour types, are essential for MA9 AML.

Conclusions: The integrated use of the model and patient AML data is allowing us to construct a more complete view of the gene expression, splicing and epigenetic changes during AML development than using patient data alone.

Outcome/Impact: Our work has established a suitable model for data-informed, hypothesis driven experiments to define the molecular mechanisms of MA9-AML and have also revealed novel potential therapeutic targets.
TFRI is an Institute without walls linking the capabilities of 64 leading cancer care and cancer research institutes and universities organized through six regional “nodes”.

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New Brunswick Health Research Foundation  
Memorial University of Newfoundland (St John’s)  
New Brunswick Cancer Network  
O’Neill Health Sciences Centre (Halifax)  
The University of New Brunswick  
The University of Prince Edward Island

TFRI is an Institute without walls linking the capabilities of 64 leading cancer care and cancer research institutes and universities organized through six regional “nodes”.

675 West 10th Ave / Vancouver / BC / Canada / V5Z 1L3  
604.675.8222 / info@tfri.ca / www.tfri.ca