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University of Alberta
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Poster Abstracts
Both 14-3-3 proteins (14-3-3s) and Rho proteins regulate cytoskeleton remodeling and cell migration, which suggests a possible interaction between the signaling pathways regulated by these two groups of proteins. Indeed, more and more emerging evidence indicates the mutual regulation of these two signaling pathways. However, all of the data regarding the interaction between Rac1 signaling pathways and 14-3-3 signaling pathways are through either the upstream regulators or downstream substrates. It is not clear if Rac1 could interact with 14-3-3s directly. It is interesting to notice that the Rac1 sequence 68RPLSYP73 is likely a 14-3-3 protein binding motif following the phosphorylation of S71 by Akt. Thus, we hypothesize that Rac1 directly interacts with 14-3-3s. We tested this hypothesis in this research. By using mutagenesis, co-immunoprecipitation (co-IP), Rac1 activity assay, immunoblotting, and indirect immunofluorescence, we demonstrate that 14-3-3s interact with Rac1. This interaction is mediated by Rac1 S71 in both phosphorylation-dependent and -independent manners, but the phosphorylation-dependent interaction is much stronger. Epidermal growth factor (EGF) strongly stimulates the phosphorylation of Rac1 S71 and the interaction between 14-3-3s and Rac1. Mutating S71 to A completely abolishes both phosphorylation-dependent and -independent interactions between 14-3-3s and Rac1. The interaction between 14-3-3s and Rac1 mostly serve to regulate the activity and subcellular localization of Rac1. Among the seven 14-3-3 isoforms, 14-3-3η, -σ, and -θ showed interactions with Rac1 in both Cos-7 and HEK 293 cells. 14-3-3γ also binds to Rac1 in HEK 293 cells, but not in Cos-7 cells. We conclude that 14-3-3s interact with Rac1. This interaction is mediated by Rac1 S71 in both phosphorylation-dependent and -independent manners. The interaction between 14-3-3 and Rac1 mostly serves to regulate the activity and subcellular localization of Rac1. Among the seven 14-3-3 isoforms, 14-3-3η, -γ, -σ, and -θ interact with Rac1.
Several classes of cancer therapies work by inducing DNA damage resulting in programmed cell death and tumor regression. Resistance to therapies however commonly develops after remission in the form of a more aggressive cancer that is not responsive to therapies. One of the proteins that facilitate this resistance is the E2 ubiquitin conjugating enzyme Ubc13, which together with its partner Mms2 build lysine 63-linked polyubiquitin chains that are precursors in both DNA damage and NF-κB signaling pathways (a pro-inflammatory and survival pathway), leading to increased survival and proliferation of cancer cells. Ubc13 has shown to drive radio-resistance in certain types of breast cancers (1) and its inhibition increased cell death of therapy-resistant diffuse large B-cell lymphomas (2), both of which are major societal burdens. These findings suggest that Ubc13 inhibitors could provide a promising novel therapy for resistant non-responsive cancer.

Ubc13’s active site pocket is distinct from all the other seventeen E2s with structures in the protein data bank this makes it an ideal drug candidate (3). We propose a novel inhibitor which shows promise in reducing Ubc13’s activity in vitro. We hypothesize it is doing so covalently by reacting with the active site cysteine of Ubc13. Preliminary data suggests the inhibitor covalently reacts with Ubc13’s active site, no reaction is observed when the cysteine is mutated to a serine. At micro molar concentrations of compound, lysine-63 polyubiquitin chain formation was halted in vitro. Additionally, a thermal shift assay suggests that the compound is binding, and at higher concentrations is stabilizing Ubc13, when compared to the cysteine mutant. Structural studies are currently on going to help us elucidate the mechanism of inhibition of this compound. Ubc13’s direct involvement in tumour cell viability makes it a very promising therapeutic target in resistant non-responsive cancers.
Cancer is heterogeneous, and multiple hallmarks, such as proliferation and survival, must be regulated simultaneously. Breast cancer as the first and ovarian cancer as the fifth cause of death in female patients, have common underlying pathways, processes, and regulatory factors. In particular, factors underlying the regulation of proliferation and apoptosis are likely similarly employed.

In this study, we first constructed protein-protein interaction (PPI) networks for breast and ovarian cancer using the STRING plugin of Cytoscape V3.7.2 and then the networks were merged together to illuminate joint nodes. Subsequently, breast and ovarian cancer microarray expression data (GSE21422 and GSE38666, respectively) was downloaded from the GEO repository and analyzed in R v3.5. The data was normalized by RMA algorithm to identify the differentially expressed genes. The expression data then was comparatively merged together to obtain a list of co-expressed common genes which were then mapped upon the joint PPI network. The genes were then subjected to the DAVID 6.7 database for functional annotation clustering and pathway analysis. The apoptotic genes were then validated using the TCGA database of both breast and ovarian cancers. The mRNA-miRNA interactions were also introduced and validated through CyTargetLinker plugin of Cytoscape and miRCancer, respectively.

There were a total of 1032 commonly expressed genes shared between breast and ovarian cancers that were filtered to 135 nodes and 888 interactions. The regulation of apoptosis was enriched in the fifth and tenth clusters. CD24, BIK, MMP9, BUB1B, KRT18, FGF2, GGCT, EPCAM, TWIST1, RPS6KA1, EFNA1, MIF, and PIK3R appear to similarly regulate apoptosis in breast and ovarian cancers. Hsa-miR-16-5p and hsa-miR-107 were the hub miRNAs which may regulate apoptosis in breast and ovarian cancers.
The Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2) protein plays an important role in signal transduction from the cell surface to the nucleus. SHP2 is a cancer driver, and its overstimulation is a key player in juvenile myelomonocytic leukemia and triple negative breast cancer. Here we focus on identifying novel drug-like molecules that inhibit the activity of SHP2 and represent validated starting points for the design of novel therapeutic agents for these conditions.

Structure-based computational database screening has been used to filter libraries of millions of small molecules for their ability to interact with the allosteric regulatory sites or the lipid-binding sites of SHP2. Thirty-eight short-listed ligands were tested for activity using recombinant SHP2 protein containing the two SH2 domains and the catalytic domain. The experiments used for cross validation included protein thermal shift (PTS), phosphatase activity, size exclusion chromatography multi-angle light scattering (SEC-MALS), and surface plasmon resonance (SPR) detection.

Eight compounds were found to consistently decreased SHP2’s activity and demonstrated binding in at least two assays. Further experiments identified two compounds that are tight binders and inhibitors of SHP2. One compound exhibited an average KD of 115 nM by SPR, which is 20-fold stronger than a known SHP2 inhibitor, NSC-87877, which exhibits a KD of 2324 nM.

This project demonstrates the importance of integrating structure-based drug design screening by computational methods and cross-validation with biophysical techniques. Efficient identification of potential inhibitors opens doors to future chemical modifications in order to improve affinity, selectivity and inhibitory action, and could lead to novel therapeutic agents in the future.
FOXQ1 is differentially expressed across breast cancer subtypes and potentially can serve as a prognostic marker

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As a member of the forkhead box (FOX) superfamily of transcription factors, FOXQ1 plays a critical role in a wide range of biological processes, including angiogenesis, epithelial differentiation, smooth muscle differentiation, mucin secretion, and helper T cell activation. Emerging evidence also show FOXQ1 to play important roles in the development and progression of various cancers such as ovarian, pancreatic, and colorectal cancer. Particularly, FOXQ1 has been linked to facilitating tumor invasion and metastasis in breast cancer and has also been specifically associated with Triple Negative Basal-like Breast Cancer (TN/BL BC).

In this study, we investigated the mechanisms leading to increased expression levels of FOXQ1 in BC through analysis of FOXQ1 copy number variation (CNV) and mRNA levels across BC patient subtypes and cell lines. Additionally, we assessed the prognostic significance of FOXQ1 in BC patients. Finally, K-means clustering was conducted by using Python coding to identify unique clusters for FOXQ1 mRNA levels and CNV in BC cell lines.

We report for the first time, that FOXQ1 mRNA is differentially expressed across BC patients. FOXQ1 mRNA is significantly down regulated in Luminal (ER+) BC patients (n=6) when compared to control samples (n=6). FOXQ1mRNA expression is significantly up regulated in TN/BL BC patients (n=6) compared to Her2 (n=6) and ER+ BC (n=6) respectively. We also found FOXQ1 significantly has more copies in TN/BL BC compared to control sample. K-means clustering analysis was conducted on BC cell lines, with the purpose of identifying distinct subpopulations based on FOXQ1 mRNA expression and CNV. Our analysis identified 3 supervised and 4 unsupervised clusters among BC cell lines for FOXQ1 mRNA compared to their CNV, however, we found FOXQ1 mRNA expression to be independent of its CNV. Moreover, FOXQ1 mRNA was found to be highly expressed in numerous TN/BL cell lines. Lastly and most importantly, by applying the bioinformatic online tool GEPIA, Kaplan-Meier survival curve identified two risk groups with high (n=524) and low (n=531) FOXQ1 mRNA expression levels. Our Kaplan-Meier survival analysis also showed patients with low FOXQ1 mRNA expression to have shorter overall survival time than those with high FOXQ1 mRNA expression (HR=0.71, P=0.042). In correspondence to these results, we propose that FOXQ1 can serve as an emerging new prognostic biomarker for BC. Understanding the mechanism(s) underlying FOXQ1’s activation in breast cancer could facilitate the development of improved therapies for BC patients.
Devin Baillie

*Medical Physics*

**High, Variable Energy Linac Design**

*Devin Baillie*

To allow treatments from 4 – 10 MV with the linac-MR system while still fitting in typical radiotherapy vaults, we designed a short, S-band, variable energy linear accelerator waveguide.

This work involved three main stages: First, an accelerator cavity was designed based on published breakdown thresholds. Second, a waveguide capable of producing 10 MV with the same length as current 6 MV waveguides was designed using this cavity geometry as its basis. Finally, tuning cylinders were added to allow the energy to be continuously adjustable from 4 – 10 MV. The waveguide was designed, tuned, and optimized using finite element method (FEM) simulations. The RF fields within the waveguide were also calculated using FEM. A particle-in-cell model was used to compute electron trajectories through the RF fields, allowing the required RF power and tuning cylinder depth to be optimized for each desired energy. Finally, Monte Carlo simulations were used to compare depth dose profiles with those produced by published electron beam characteristics for Varian linacs at 4, 6, 8, and 10 MV.

For each photon energy, the depth of maximum dose matched that produced by the Varian linac within 1.5 mm, and the ratio of dose at 10 cm depth to 20 cm depth matched within 1%. Additionally, the simulated waveguide has a beam current of 133.7 mA at 10 MV, compared to that on a Varian linac of 40 mA. This increased beam current will allow higher dose rates, but may also present practical issues during operation.
Background: Primary sclerosing cholangitis (PSC) is a chronic, cholestatic liver disease characterized by multifocal biliary strictures usually involving the intrahepatic and extrahepatic biliary tracts. PSC is found to be associated with an increased risk of malignancy in the bile ducts, gallbladder and colon. Currently, there is neither an obvious pathogenesis nor an effective treatment for PSC and high proportion of these patients undergo liver transplant or death, eventually.

Aim: To investigate the pathogenesis of PSC.
Method: Evaluation of whole genome expression profile using public microarray databases.
Results: We found several dysregulations in expression of the genes in PSC patients compared to healthy controls, mostly related to viral activity in the cell, including Apolipoprotein B mRNA Editing Catalytic Polypeptide-like enzymes (APOBECs). APOBECs are involved in innate immune response to retroviral infection.
Conclusion: We investigated a possible retroviral etiology of the PSC. Our next goal is to find the retrovirus sequence and using antiretroviral therapies, subsequently.
Marina Bianchi Lemieszek

Obstetrics and Gynecology

The role of the Epithelial Splicing Regulatory Protein 1 (ESRP1) in Luminal A Breast Cancer cell lines.

Marina B. Lemieszek, Scott S. Findlay, Krista Vincent, Lynne-Marie Postovit

Introduction:
Breast cancer is the most common cancer among Canadian women and has different molecular subtypes. Luminal A subtype shows the most favorable 5-year prognosis; however, its recurrence is responsible for a high increase in mortality after 10 years of diagnosis. Therefore, it is important to identify prognostic markers that predict breast cancer recurrence. The Epithelial Splicing Regulatory Protein 1 (ESRP1) is an RNA binding protein that regulates alternative splicing events associated with epithelial cell types. High levels of ESRP1 correlate with reduced patient overall and disease-free survival. Thus, ESRP1 may have a pro-metastatic role in breast cancer. This project goal is to determine the function of ESRP1 in two luminal A breast cancer cell lines.

Methods:
Two luminal A breast cancer cell lines, MCF7 and T47D, were edited using CRISPR gene editing to knockout ESRP1 gene. These cells were screened for frameshift mutations with Droplet Digital-PCR (ddPCR) and mutations are being confirmed with Sanger Sequencing. The ESRP1 knockout cells and controls will be used to determine in vitro growth rate and to perform Mammosphere Formation assay. Expression of Epithelial-to-Mesenchymal transition markers will be analyzed with Real Time-PCR and with Western Blot. Additionally, breast cancer samples from one hundred patients that had breast cancer recurrence and from one hundred patients that did not recur will be analyzed with ddPCR to determine the copy number of ESRP1 gene.

Results:
ESRP1 knockout and wild-type clones identified with ddPCR are being screened for mutations. CRISPR was done twice in T47D cell line and clones were seeded in Matrigel to help in cell attachment. Copy number of ESRP1 gene is being analyzed in MCF7 and T47D cell lines.

Conclusion:
CRISPR gene editing was performed in two breast cancer cell lines. Breast cancer samples will be analyzed to determine the copy number of ESRP1 gene.
Olena Bilyk

Oncology

Embryonic Protein Nodal as a Potential Marker of Drug Resistance and Recurrence in Ovarian Cancer

Olena Bilyk, Laura Lee, Dylan Dieters-Castator, Nhu D. Le, Linda Cook, Martin Koebel, Lynne-Marie Postovit

Ovarian cancer (OC) is the most aggressive gynaecological cancer due to the high rate of chemoresistant recurrence. Aggressive cancer cells can exploit normally dormant embryonic stem cell pathways to promote cancer cell plasticity and tumor recurrence following chemotherapy. Studying embryonic signalling pathways in aggressive cancers has led to the discovery of the re-expression of the embryonic protein Nodal, the member of TGF-β family of proteins. The objective of this study is to investigate the role of Nodal as a potential biomarker of ovarian cancer (OC) cell plasticity, progression and resistance to chemotherapy.

Methods. We applied bioinformatics approach, RNA sequencing and mass spectrometry (MS) to explore the impact of Nodal on OC cells and disease outcome. We conducted in vitro assays designed to assess growth, cancer stem cell like phenotypes and chemoresistance in OC cells with different sensitivity to platinum, wherein Nodal was added with Nodal expression construct, or knockdown with shRNA. IHC staining to evaluate Nodal expression was conducted in 563 high-grade serous OC (HGSOC) tissue microarrays (OVAL-BC study cohort). Survival analysis was performed based on Nodal expression in tissue microarrays in OVAL-BC study cohort and TCGA data.

Results. In vitro, in OC cells sensitive to chemotherapy, Nodal expression significantly increased resistance to cytostatic drugs (platinum and taxol), tumorigenicity, cancer cell plasticity (EMT, cancer stem cell like phenotype), and disrupted the expression of cell cycle control protein p21WAF1/CIP1. Resistant to platinum OC cells didn’t display significant difference in carboplatin sensitivity and tumorigenicity in vitro when Nodal was overexpressed.

By RNA sequencing and MS analysis we discovered that Nodal induces transcriptional reprogramming in OC cells, sensitive to chemotherapy, via altering immune and inflammatory response, metabolism and drug resistance gene expression. More than 3000 genes were up- or down-regulated in response to Nodal overexpression in chemotherapy sensitive OC cells.

In univariate Kaplan-Meier analysis, high Nodal expression was significantly associated with shorter OC specific survival. HGSOC patients with high Nodal expression were less likely to achieve complete debulking after surgery compared to patients with low Nodal expression.

Conclusion. Nodal predicts poor survival and aggressive phenotype in patients with most aggressive high-grade serous OC. Nodal likely drives tumorigenic potential and resistance to platinum and taxol in OC cells, sensitive to chemotherapy, by promoting cancer cell plasticity and upregulating target genes involved in immune suppression, inflammation, drug resistance and metabolism. Nodal may hold a promise as a therapeutic target to prevent OC recurrence following chemotherapy.
Selective ablation of cancer cells with dysfunctional p53 signaling using novel suicide gene approach

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The tumor suppressor p53 is frequently mutated or dysregulated in cancer, and as a result the upstream signaling pathways activating p53 transcription are strongly upregulated. RNA-seq analysis has demonstrated that p53 transcription is significantly upregulated in nearly all forms of cancer. Additionally, HCT116 cells lacking functional p53 display a ~6-fold increase in p53 promoter activity when compared to its wild type p53 parent cell line. To exploit this upregulation, we have developed a p10 plasmid vector to deliver the p53 promoter-driven suicide gene, inducible caspase-9 (p10-p53-iCasp9), to tumor cells and destroy them upon treatment with a small molecule chemical inducer of dimerization (CID). A robust induction of cell death following transfection with p10-p53-iCasp9 and sequential CID administration was observed in cancer cells, as measured using cell viability assays, imaging assays, and Annexin V and TUNEL flow cytometry. Induction of iCasp9 protein expression and caspase-mediated apoptosis was confirmed using Western blot. In order to systemically deliver p10-p53-iCasp9 we also developed a Fusogenix lipid nanoparticle (LNP) formulation. Fusogenix LNPs demonstrated efficient delivery of plasmid DNA in immune-competent mice as determined via bioluminescent imaging. The anti-tumor efficacy of our Fusogenix LNP p10-p53-iCasp9 formulation was evaluated in a xenograft H1299 model. Subcutaneous tumors were grown in the flanks of immune-compromised mice until tumors were palpable before intravenous LNP injection and subsequent CID administration occurred. We detected iCasp9 expression exclusively in H1299 tumors. Furthermore, tumor volume was dramatically stabilized relative to control animals when p10-p53-iCasp9 and CID was delivered. Apoptosis was confirmed via TUNEL staining on tumor sections. We describe here a novel Fusogenix LNP gene therapy approach for the treatment of cancer with a selectivity for tumors with dysregulated p53 transcriptional activation.
We used the cancer intrinsic property of oncogene-induced DNA damage as the base for a conditional synthetic lethality approach. To target mechanisms important for cancer cell adaptation to genotoxic stress and thereby to achieve cancer cell-specific killing, we combined inhibition of the kinases ATR and Wee1. Wee1 regulates cell cycle progression, whereas ATR is an apical kinase in the DNA damage response. In an orthotopic breast cancer model, tumor-selective synthetic lethality between bioavailable ATR and Wee1 inhibitors led to tumor remission and inhibited metastasis with minimal side effects. ATR and Wee1 inhibition had a higher synergistic effect in cancer stem cells than in bulk cancer cells, compensating for the lower sensitivity of cancer stem cells to the individual drugs. Mechanistically, the combination treatment caused cells with unrepaired or under-replicated DNA to enter mitosis leading to mitotic catastrophe. As these inhibitors of ATR and Wee1 are already in phase I/II clinical trials, this knowledge could soon be translated into the clinic, especially as we showed that the combination treatment targets a wide range of tumor cells. Particularly the anti-metastatic effect of combined Wee1/ATR inhibition and the low toxicity of ATR inhibitors compared to Chk1 inhibitors has great clinical potential.
Transcription factor ZIC2 promotes tumorigenic phenotypes by regulating the biology of the bulk and cancer stem cells in ovarian cancer

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Objectives
Epithelial ovarian cancer (EOC) is the leading cause of gynecological cancer death in women. Current therapeutic regimen is ineffective in killing cancer stem cells (CSCs). A combined therapy that targets both bulk cells and CSCs would be a more effective therapeutic strategy to manage and/or prevent recurrent and resistant disease. Transcription factor ZIC2 has emerged as an oncogenic factor in many human cancer. This project aims to investigate the pro-tumorigenic role of ZIC2 in EOC and the underlying mechanisms.

Methods
The EOC patient survival and ZIC2 expression from The Cancer Genome Atlas (TCGA) were analyzed for correlation. ZIC2 expression was examined in a panel of EOC cell lines at protein level. ZIC2 knockout via CRISPR or overexpression models were generated in EOC cell lines. The effect of ZIC2 knockout or overexpression on gene expression (quantity RT-PCR, RNA-sequencing, Western blotting and immunohistochemistry (IHC)), the percentage of CSCs (Western blotting and ALDEFLUORTM assay), growth (neutral red uptake assay), migration (Transwell migration assay), anchorage-independent growth (soft agar assay), self-renewal (limiting dilution sphere formation assay), and tumor formation (subcutaneous xenograft model) was examined in EOC cells.

Results
Higher ZIC2 mRNA expression is associated with shorter survival of EOC patients (TCGA analysis). Knockout of ZIC2 in EOC cell lines results in decreased cell growth, migration, ALDHhigh population and sphere-forming ability, and tumor formation in the mouse subcutaneous xenograft model. ZIC2 knockout dramatically down-regulated the expression of genes critical for multiple biological processes, including CSC, cell cycle, EMT, signaling kinases, invasion and metastasis, and mRNA binding. Western blotting and IHC confirmed that ALDH1A1 and cyclin D2 were only expressed in the ZIC2 wild-type, but not in ZIC2 knockout, EOC cells-derived xenografted tumors.

Conclusions
Our work provides insights that ZIC2 significantly promotes tumorigenic phenotypes through maintaining CSC population in EOC.
Human B7-1 (CD80) is a member of the B7 family, which plays a key role in regulating T-cell function. B7-1 is normally expressed as a homodimer on the surface of antigen-presenting cells. It interacts with two T cell receptors, namely CD28 and CTLA-4 to deliver co-stimulatory and co-inhibitory signals, respectively. Thus, B7-1 is related to the treatment of both autoimmune disease and cancer. Since B7-1 is involved in different immunoregulatory functions, it has been considered as an important immunotherapy target, leading to the discovery of several B7-1 small molecule inhibitors. However, neither the sites nor the modes of binding for these molecules were clearly characterized. In this project, we aim to use computational modeling tools to investigate the binding site(s) and mode(s) of action of a potent B7-1 small molecule inhibitor. We modeled the B7-1 protein in both monomer and homodimer states after adding a missing c-terminal domain and glycans. The two models were solvated in a TIP3P water box and Na+ and Cl− ions were added to achieve an electrostatically neutral environment. The solvated systems were energy minimized and equilibrated. Up to 100ns molecular dynamics (MD) simulations were performed to obtain stable conformations of B7-1. Clustering analysis was then performed to select representative conformations for docking analysis. Flexible docking was then used to identify the most probable binding locations and modes of binding of the B7-1 inhibitor. Based on our MD simulations, the B7-1 dimer showed more stability than the monomer. Potential binding poses of inhibitor:B7-1 were also identified showing low binding energy and physicochemical complementary. Through this project, the B7-1 models were established. MD simulations and docking analysis identified the most probable mode of binding of the small molecule B7-1 inhibitor. Further analysis, including free energy calculations on the generated complexes are required to confirm these findings.
Introduction: The development of trastuzumab has greatly improved clinical outcome for HER2+ breast cancer patients. However, intrinsic and acquired resistance to trastuzumab are common, with cancer stem cells and epithelial-mesenchymal-transition (EMT) known to drive resistance to trastuzumab treatment.

Retinoic acid (RA) is a vital signalling molecule that regulates cell proliferation, differentiation, and death. RA is used for the treatment of some leukemias. The HER2 gene is frequently co-amplified with retinoic acid receptor alpha (RARalpha), a key determinant of RA sensitivity in breast cancer. Unexpectedly, HER2+ breast cancers are refractory to RA treatment for unknown reasons.

MYC has been shown to interact with RARalpha, thereby suppressing RA-responsive genes and differentiation. Furthermore, MYC upregulates FABP5, an RA binding protein that delivers RA to nuclear receptor PPARbeta resulting in pro-tumorigenic effects. Hypothesis: MYC attenuates RA action in HER2+ breast cancer cells.

Results: We found that MYC is preferentially amplified in HER2+ breast cancers. MYC inhibits RAR transcription activity in HER2+ breast cancer cells. These results, along with results showing that MYC attenuates RA-induced inhibition of cell proliferation, suggest that MYC plays a major role in RA action in HER2+ breast cancer cells. Notably, RA inhibits EMT in cells with low MYC expression, pointing to possible synergy between RA and trastuzumab, as EMT drives trastuzumab resistance.

Conclusions: MYC attenuates the anti-cancer effects of RA by inhibiting RAR activity in HER2+ breast cancer cells. Further studies will focus on investigating the role and mechanism of MYC in governing RA resistance in HER2+ breast cancer cells.
Purpose: Brachytherapy (BT), a boost treatment following external beam radiotherapy (EBRT) for locally advanced cervical cancer, can be delivered in high-dose-rate (HDR; daily/weekly fractions) or pulsed-dose-rate (PDR; hourly pulses over a few days) regimens and is prescribed using radiobiological dose. Current radiobiological dose calculations use radiobiological parameters $\alpha/\beta$ and $T_1/2$. Conventional values of both $\alpha/\beta$ (10 Gy for tumours) and $T_1/2$ (1.5 hours) have been assumed in cervical cancer BT prescription without precise justification except for a lack of contradictory clinical evidence. However, significant variation exists in the numeric value of these in the literature. This study was conducted to determine the potential impact of these variations on BT dose calculations.

Materials/Methods: A literature search was performed for radiobiological parameter values of cervical cancer tissue. To quantify the effect of the reported $\alpha/\beta$ and $T_1/2$, the radiobiological tumour dose was calculated for six potential BT boosts following EBRT (25 fractions of 1.8 Gy each) using previously established radiobiological dose equations and dose prescription units (EQD2; EQuivalent Dose in 2 Gy fractions). All potential schedules would deliver a tumour dose of approximately 90 Gy EQD2 (combined EBRT and BT) using conventional $\alpha/\beta$ and $T_1/2$ values.

Results/Conclusion: The literature reported an $\alpha/\beta$ range of 6-21 Gy and $T_1/2$ range of 0.25-0.64 hours for cervical tumour tissue. These values were based on patient outcomes studies and in vitro experiments. Based on the radiobiological dose calculations, deviation of either parameter from conventional values could result in non-equivalent radiobiological doses being delivered by the PDR and HDR BT boosts that are assumed to be conventionally dosimetrically equivalent. The difference in treatment schedules could be over 9 Gy in EQD2 given the reported values, which could have potentially significant clinical consequences. This highlights the need for further efforts to establish more definitive parameter values.
One of the most lethal yet rare subsets of uterine cancer is dedifferentiated endometrial carcinoma (DDEC). Less than 20% of patients diagnosed with DDEC survive compared to the over 80% of uterine cancer patients with high-grade endometrial cancer diagnoses. DDEC tumors possess both well-differentiated and undifferentiated regions. Previously, we demonstrated that 80% of the undifferentiated regions in DDEC lesions lack the expression of core chromatin remodeling proteins, SMARCA4 or ARID1A and ARID1B. We hypothesize that loss of these proteins, which are known regulators of transcription may lead to the induction and/or maintenance gene expression programs that drive dedifferentiation, metastasis and therapy resistance.

SMARCA4-deficient endometrial cancer (EC) cell line models were generated by CRISPR gene editing and were found to be less capable of self-renewal and anchorage-independent growth. In vitro, SMARCA4 knockout cells were found to be more senescent than their wild-type counterparts, possessing more positive beta-galactosidase stained cells and expressing higher levels of p21 and H3K9me3. Tumors formed from SMARCA4-deficient EC cell line models in immune-compromised mice recapitulated the mixed phenotype observed in patient DDEC lesions. Endometrial cancer cells lacking SMARCA4 expression were also found to be more sensitive to inhibition with clinically available therapeutics targeting CDK4 and EGFR. Synergistic effects upon combining therapies against CDK4 and EGFR will be evaluated. Exome sequencing, RNA-Seq, ChIP-Seq and ATAC-Seq will be carried out to elucidate whether it is the consequence of acquired mutations, changes in gene expression or alterations to nucleosome occupancy that contribute to cellular dedifferentiation in the context of DDEC.
Reovirus is a nonpathogenic virus that naturally inhabits the intestine of humans. Reovirus can also selectively replicate in tumor cells and is therefore a candidate for cancer therapy undergoing clinical trials. Given that wild-type reovirus (T3wt) is naturally adapted to intestinal environment (rather than tumors), I hypothesize that reovirus can be modified to infect tumor cells more efficiently. First, by using plaque size to reflect the proficiency of reovirus replication in tumor cells, I tested 24 reovirus point mutations and I discovered that eight of them increased the oncolytic potency relative to T3wt. With the idea of creating the most-oncolytic reovirus, I found that the combination of oncolytic mutations have additive effects on oncolysis in vitro.

Then, I identified that five of the eight oncolytic mutations have mechanisms of oncolysis that have not been previously described. One of the mechanisms is the increased apoptosis induction. In addition, I selected the best individual and combined mutants in vitro to test them in vivo. In three different immunocompetent mouse models of breast tumor, I have established the optimal dosing and scheduling of T3wt to observe tumor reduction and increased survival. Currently, I am evaluating the oncolytic potency of the best reovirus variants in the optimized models of breast tumor. In future experiments, I will determine how mutations in reovirus can promote direct virus-induced killing of cancer cells and/or anti-tumor immune responses. My experiments will provide a better understanding of reovirus biology and how to improve reovirus as a cancer therapy.
Introduction: Granulosa cell tumor (GCT) is a malignant sex-cord stromal cell form of ovarian cancer that constitutes ~5% of ovarian neoplasms. Current chemotherapy regimens are not effective enough in controlling recurrent GCT and ~80% of women who relapse will die of disease. Procaspe-activating compound 1 (PAC-1) is a small-molecule activator of procaspe-3 that has been extensively studied both in vitro and in vivo. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) has failed to replicate in vitro efficacy in the clinic due to inadequate delivery methods; a short half-life; and inherent or acquired resistance to TRAIL therapy. Vaccinia virus (VACV) is an ideal vector for use in viral gene therapy. It has a short lifecycle, rapidly spreads cell-to-cell, has strong lytic ability, and a large cargo capacity. Here we report on the development of a recombinant, tumour-selective VACV expressing TRAIL (VACV-TRAIL) that generates effective levels of soluble TRAIL during the viral replication cycle and combines with PAC-1 to control GCT, in vitro.

Results: PAC-1 combination with TRAIL displays synergy in KGN cells with strong activation of caspase-3 within 24 hr of treatment. Importantly, PAC-1/TRAIL combination is similarly effective on patient-derived GCT cells while being much less cytotoxic to normal cells. VACV-TRAIL replicates effectively in KGN and virus-mediated cytotoxicity is greater than that of VACV WT, and is potentiated by combination with PAC-1. VACV-TRAIL generates effective levels soluble TRAIL that combined with PAC-1 controls KGN cells as well or better than rhTRAIL.

Conclusions: To our knowledge, this is the first attempt to combine oncolytic vaccinia gene therapy with PAC-1. VACV-TRAIL is an efficient vector for gene therapy and combined with PAC-1 effectively controls GCT, a disease with few current treatment alternatives. The combination provides a three-pronged strategy for cancer control through activation of caspase-3, increased death signaling, and self-amplifying replication of oncolytic virus.
External beam radiotherapy involves the use of a linear accelerator (linac) to generate high energy radiation to destroy cancerous tumours. Real-time adaptive radiotherapy requires continuous imaging throughout treatment to track and monitor the tumours location during treatment. Current clinical linac systems do not have the ability to image during treatment, requiring larger treatment volumes to ensure the tumour is receiving the prescribed radiation dose. Unfortunately, this also damages surrounding healthy tissue making treatments with tumours located in close proximity to sensitive organs difficult. Tracking a tumour in real-time may allow for a decreased dose to sensitive organs and healthy tissue surrounding the tumour by minimizing the treatment margin within the planning target volume. Adaptive radiotherapy is ideal for tumours situated within or nearby organs of motion, such as the lung, due to the potential for reduced treatment margins that are conventionally used to compensate for motion. Our team has recently developed of a hybrid radiotherapy system that combines a linac with magnetic resonance imaging (MRI), which will have the capability for real-time MR guided adaptive radiotherapy. The use of MRI is ideal for real-time imaging: aside from the excellent soft tissue contrast, it does not contribute any additional dose to the patient during imaging. Unfortunately, real-time MRI with low latency is difficult to achieve. By accelerating the imaging, an increased amount of artefacts will be present in the reconstructed image. We have recently developed a technique that utilizes patient-specific convolutional neural networks (CNNs) to enable real-time MRI. We quantitatively verified the technique using six non-small cell lung cancer patients, which were retrospectively undersampled. The tumour segmentations from the CNN reconstructed data were compared to the fully-sampled data, which resulted in an average Dice coefficient of 0.9 or greater. Furthermore, the technique was tested using prospectively undersampled data from a volunteer, which yielded qualitatively acceptable images.
MicroRNAs (miRNA) are short (~22 nt) single-stranded RNA that is associated with the development of diseases including cancers. Fast and sensitive detection of miRNAs are of great importance for understanding their functions and using them as biomarkers. Cas13a is a Class 2 type VI-A CRISPR-associated protein that has an RNA-guided RNase function. Cas13a has been used for detection of pathogens. The objective of this research is to develop an amplification assay, facilitated by Cas13a, for fast and sensitive detection of a specific miRNA. We first expressed Cas13a protein in E.coli and purified it with nickel column, and obtained 28 µg Cas13a from 100 mL of bacteria cells, with a purity of 79%. The Cas13a protein was then used to react with crRNA, forming a Cas13a/crRNA complex. In the presence of a specific miRNA target, hybridization of the miRNA with crRNA initiates the RNase activity of Cas13a. Consequently, the activated Cas13a cleaves a RNA reporter that is dually labeled with a pair of fluorophore and quencher. Therefore, a single miRNA molecule leads to cleavage of hundreds of the reporter molecules, achieving fluorescence signal amplification. The assay was able to detect as low as 0.1 pM miRNA target within 30 min. A dynamic range from 0.5 pM to 1000 pM was achieved. In addition, the assay is conducted in a mix-and-read format under room temperature, which is promising for on-site analysis and point-of-care testing.
Katherine Ford

Agricultural, Food and Nutritional Science

Characterizing Body Composition, Energy Expenditure, Dietary Intake, and Physical Function in People with Newly Diagnosed Colorectal Cancer (CRC)

Ford KL, Trottier CF, Sawyer MB, Prado CMM

Background: Optimal nutrition is essential for cancer survivorship, yet malnutrition continues to negatively impact people battling this disease. Depleted muscle mass is evident in people with cancer and indicative of derangements in energy balance. However, energy expenditure, dietary intake and physical function have not been well characterized in these patients. Objective: To profile body composition, energy expenditure, dietary intake, and physical function in people with newly diagnosed stage II-IV CRC. Methods: Patients were recruited from the Cross Cancer Institute (Edmonton, AB). Body composition was assessed by quantifying appendicular skeletal muscle mass index by dual energy x-ray absorptiometry, and low muscle mass defined according to previously published cutoffs. Resting energy expenditure (REE) was measured by indirect calorimetry and hypermetabolism was defined as REE>110% of predicted values. Dietary intake was assessed by three-day food record and physical function by the Short Performance Physical Battery (SPPB) test. Descriptive analysis was performed to provide a cross sectional profile of this cohort. Results: Thirty-two participants (age=58.3±10.2 years; BMI=27.4±5.7 kg/m2, 53.1% males) were included. The predominant tumor site was the colon (84.4%). Most participants (59.4%) had stage 3 disease. Low muscle mass at diagnosis was observed (21.9%). More than half of participants (n=18) were hypermetabolic. Dietary data was available for a subset of participants (n=26) and found 50.0% and 53.9% did not meet minimum recommended calorie (25 kcal/kg body weight) and protein (1.0 g/kg body weight) intakes, respectively. Poor physical performance was observed in 28.1% of participants. Conclusion: Low muscle mass, hypermetabolism, inadequate dietary intake, and poor physical performance were observed across stages of patients with newly diagnosed CRC, reflecting the need for nutritional interventions at the start of chemotherapy treatment. Future research should investigate impacts of treatment on these outcomes and aim to develop targeted nutrition recommendations for this population.
DEAD box proteins are RNA unwinding proteins that play important roles in RNA metabolism. DEAD box 1 (DDX1) was first identified in our laboratory and subsequently found to be overexpressed in neuroblastoma, retinoblastoma and breast cancer. DDX1 is primarily found in the nucleus of most proliferating cells and is also abundant in the cytoplasm of cells overexpressing the protein. Nuclear DDX1 promotes the repair of DNA double strand breaks induced by genotoxic stress. When cells are exposed to environmental stressors, such as oxidative stress and endoplasmic reticulum stress, DDX1 re-localizes to cytoplasmic stress granules (SGs). Although DDX1 is dispensable for SG assembly, depletion of DDX1 leads to impaired SG disassembly and hence, delayed stress recovery. We postulate that DDX1 plays a key role in oxidative stress recovery in neuronal cells.

Using RNA-DDX1 immunoprecipitation and next generation sequencing, we have identified several DDX1-bound RNAs in cells cultured under normal and stress conditions. My goal is to examine the importance of RNAs whose binding efficiency is altered under stress condition. To address this question, I will use the neuroblastoma cell line SH-SY-5Y. These cells can be induced to differentiate into neuronal-like cells with retinoic acid. I will examine levels and localization of selected RNAs in undifferentiated and differentiated SH-SY5Y cells cultured under normal and oxidative stress conditions. Next, I will compare wild type and DDX1-depleted SH-SY-5Y under normal and stress conditions to directly address the role of DDX1 in neuronal stress response. Finally, I will investigate the role of DDX1 in regulating the translation of its bound RNAs during stress and at the recovery stage in SH-SY-5Y cells. This study will provide insight into the role of DDX1 in oxidative stress response and set the stage for a better understanding of both brain tumours and neurodegenerative diseases.
Ocular plaque brachytherapy is an effective treatment option for medium-sized ocular melanomas, showing equivalent survival relative to enucleation, while providing added benefits of eye preservation and possible vision retention. Currently the treatment planning system for plaque brachytherapy, known as Plaque Simulator, generates treatment plans using uniform seed strengths which requires the planner to modify the plan by manually adjusting the seed strengths to reduce doses to the critical structures while maintaining the desired tumour coverage. This demands planning expertise, can be time-consuming, and may not always provide the best possible plan. To overcome these challenges, my work applies an automated dose optimization algorithm, known as simulated annealing algorithm (SAA), to plan plaque brachytherapy treatments. Treatment plans were generated using COMS plaques (diameters from 12 to 22 mm) for treating tumours of various dimensions (base and height). An optimization routine using the SAA was generated with Matlab (v2017b, MathWorks, Natck MA) to determine optimal strength for each seed used in the plaque so as to minimize doses to the optic disc (OD) and fovea, while delivering the prescription dose to the entire tumour. The target dose for OD and fovea were set to 60 and 50 Gy, respectively, with equal weights. For a 5 mm tall tumour with 12 mm plaque, optimized plans resulted in a change in dose to OD and fovea compared to using conventional uniform loading of -24.0 ± 0.4% and -28.4 ± 0.1%, respectively. Similarly, for an 8 mm tall tumour with 16 mm plaque, -37.3 ± 1.6% and -39.6 ± 0.2% reductions were achieved and for a 3.5 mm tall tumour with 22 mm plaque, +0.7 ± 0.2% and -0.8 ± 0.3% were achieved. Moreover, by applying the algorithm to numerous clinical scenarios, it was verified that implementing the developed optimization routine into clinical cases is practical.
Karen Hoy

Oncology

Overview of analytical considerations for arsenic speciation analysis

Karen S. Hoy, Xiufen Lu and X. Chris Le

Exposure to elevated concentrations of arsenic is associated with a greater risk for developing cancers, cardiovascular diseases, and diabetes. Arsenic naturally occurs in the environment and can enter water bodies from weathered minerals. Additionally, many anthropogenic releases - such as pesticide application, coal and wood combustion - contribute to elevated arsenic concentrations. Arsenic species have varying toxicities. For example, high abundances of arsenobetaine are relatively non-toxic and rapidly excreted in urine; however, chronic consumption of elevated concentrations of inorganic arsenic is known to be associated with skin, bladder, and lung cancers. Therefore, it is critical to distinguish between and determine the concentration of arsenic species in various samples, including food, water, and urine, to assess consumption risks. To detect and quantify arsenic species, the most common instrumentation method used is high performance liquid chromatography (HPLC) for separation followed by inductively coupled plasma mass spectrometry (ICPMS) for detection. Although the same instrumentation can be used for arsenic speciation of a variety of samples, modifications to sample preparation and instrument methods are required to achieve a robust method and obtain accurate data of arsenic species concentrations. Explanation of key analytical considerations using an HPLC-ICPMS for arsenic speciation analysis derived from laboratory experience and literature will be presented. Considerations examined include appropriate extraction methods for solid samples, choice of column, type of arsenic species of interest, selecting appropriate reference materials, and confirmation of arsenic species identity. Fish and urine have distinctly different matrices and often contain different abundances of arsenic species. These two samples are used as examples to understand analytical considerations for arsenic speciation analysis. Understanding how to select for sample preparation, analysis methods, and reference materials, as well as how these parameters affect the data collected, will aid in creating an optimal method for arsenic species quantification for any desired sample.
Cardiovascular complications as a result of cancer therapy is a growing challenge for many health care systems. This rising concern is also reflected in the drug development arena as the frequent emergence of cardiotoxicity from many approved anticancer treatments led to either discontinuing their use or, in some cases, their withdrawal from the market. A profound example is the class of tyrosine kinase inhibitors (TKIs), where there is a desperate and unmet need for strategies to eliminate their off-target cardiotoxic effects. However, the mechanisms for their induced cardiotoxicity are not fully understood and are relatively unexplored. Here we focused on understanding the mechanism of interaction of several TKIs with the hERG channel by combining multiscale molecular modelling and biochemical experiments. The modeling workflow employs various in silico structure-based approaches and provides qualitative and quantitative insights into TKIs binding to hERG at the molecular level. The eight TKIs, gefitinib, lapatinib, sunitinib, vandetanib, crizotinib, nilotinib, ruxolitinib, vemurafenib, were tested in comparison to the standard E-4031 (IC$_{50}$ ≈ 115 nM) and were shown to possess varying levels of activity, ranging from IC$_{50}$ of ~8.6 to 159.1 μM in a hERG fluorescence polarization assay. The modeling protocol helped us differentiate the structurally similar analogs and understand the binding mechanism of these compounds to the hERG channel. The structural analyses also indicate that the changes in the structural networks and the disruption of the native π-π network present in the channel lead to their varying activities against hERG. The combined results of these two interrelated methodologies provide a comprehensive understanding of the cardiotoxic mechanism of action of TKIs and sheds light on the
Human polynucleotide kinase/phosphatase (PNKP) plays a key role in DNA repair by processing 3’-phosphate and 5’-hydroxyl strand break termini. Mutations in PNKP are known to cause microcephaly, seizures, and developmental delay (MCSZ), a serious neurodevelopmental disorder. Recently, a child with MCSZ presented at Seattle Children's Hospital with a cerebellar glioblastoma multiforme (GBM). The child was shown to have two germline mutations in PNKP, which causes P101L and T323M mutations in PNKP protein. This is the first report of human cancer in a patient with germline PNKP mutations. Furthermore, these mutations have not previously been found in any reported MCSZ patients. The focus of this study is to try to understand the molecular consequences of these specific PNKP mutations and the functional changes to PNKP protein at the molecular level. We generated the mutant PNKP constructs in both bacterial and mammalian expression vectors. The bacterially expressed mutant proteins as well as the wild type were purified and tested with kinase and phosphatase assays. The results showed that both kinase and phosphatase activities of mutant PNKPs were reduced. We also transfected HeLa PNKP knockout cells with wild type and mutant GFP-PNKP constructs. High content analysis and confocal microscopy showed a greater presence of the mutant PNKP in the cytoplasm compared with wild type while inhibition of nuclear export protein CRM1 changed the localization pattern in one of the PNKP mutants. In addition, the DNA repair ability of mutant PNKP transfected cells were also reduced compared to wild type.
The cytosolic protein, Trex1, is induced at a certain radiation dose threshold in different cancer cells, and its induction weakens the immunogenic potential of the cells by degrading radiation-induced single-stranded (ss) or double-stranded (ds) DNA fragments that accumulate in the cytoplasm. ss/ds-DNA breaks induced by ionising radiation frequently contain non-conventional termini, including 3’-phosphate (3’-P) and 5’-hydroxyl groups requiring further processing by the enzyme polynucleotide kinase/phosphatase (PNKP) prior to DNA ligation. Interestingly, Trex1 degrades ss/ds-DNA from the 3’-end. However, its activity is blocked by DNA termini harbouring 3’-obstructive groups, such as 3’-P. Because radiation-induced DNA ss/ds break termini frequently contain 3’-phosphates, we hypothesise that, in vivo, TREX1 may potentially require the phosphatase activity of PNKP in order to degrade cytosolic DNA. Consequently, the absence of PNKP would stall TREX1 activity, stabilise cytosolic DNA and subsequently potentiate the cGAS-STING pathway and immunogenic response following exposure of cancer cells to irradiation.

In support of our hypothesis, we have found: (1) PNKP-deficient HCT116 (colorectal cancer cells) exposed to IR have elevated levels of STAT1 phosphorylation (an indication of cGAS activation) compared to wild type HCT116 cells, (2) PNKP is present in micronuclei of irradiated wild type cells, and (3) PNKP-deficient HeLa cells display significantly more cytosolic DNA than wild type cells.

In the next year, we are proposing to use a variety of techniques to achieve our aims to (1) understand the potential role of PNKP in the cGAS-STING pathway and (2) isolate, characterize and delineate the nature of cytosolic ss- and ds-DNA generated following irradiation. Results from this study will provide significant insights into the relationship between a DNA repair protein, PNKP, and the cGAS-STING pathway, as well as provide further support for the development of an inhibitor against the PNKP enzyme for clinical applications in combination therapy with ionizing radiation against tumours.
Deoxyribonucleic acid (DNA), a molecule necessary for an organism’s basic survival is prone to damage due to various cellular and environmental factors. Cells have a critical defense machinery against the re-occurring damage, necessary for maintaining genomic integrity and prevention of toxic consequences such as carcinogenesis. In DNA damage response (DDR), a cascade of signaling events occur where either the repair pathway is activated, or apoptosis is initiated if repair is impossible.

The genomic DNA is wrapped around an octamer of four core histones: H2A, H2B, H3 and H4. The exposed positive charges stabilize the octamer-DNA interaction. Together, nucleosome has a compact histone core and eight lysine-rich flexible histone tails that binding sites for various non-histone proteins. In humans, H2AX, an H2A histone variant exists, which is necessary for retention of DDR factors. This variant has a C-terminal motif that is phosphorylated by ATM kinase on serine 139 (forming γH2AX), an indication of DNA double strand break (DSB) recognition. This leads to the recruitment of an intricate network of chromatin modifying proteins to the DSB site.

Mediator of DNA damage checkpoint protein 1 (MDC1) is an early player in the DDR pathway and their knockout phenotypes present significant genomic instability. In the early stages of DDR, the BRCT domain of MDC1 specifically interacts with the phosphorylated C-terminal tail of γH2AX nucleosome. This BRCT domain is structurally conserved across various DNA damage involved proteins and presents remarkable specificity to binding only the pSxxY-COOH sequence. The details of this interaction in the context of chromatin is yet to be explored. We have recombinantly expressed and purified in-vivo phosphorylated serine 139 nucleosomes and aim to provide insights into the dynamics of MDC1-nucleosome interaction. The study will elucidate nucleosome dynamics during DDR, thus providing targets for therapeutic intervention.
Validation of a role for Active Beta-Catenin (ABC) in Promoting Metastatic Phenotype in Osteosarcoma

Takaaki Landry, Noureen Ali, Elizabeth Garcia, Jonathan Bush, Danielle Cohen, Rebecca Deyell, Mary Hitt, David D. Eisenstat, Sujata Persad

Introduction: Osteosarcoma is an aggressive primary bone malignancy with peak incidence in children/young adults. The 5-year event-free-survival for patients with localized disease is ~65% and ~25% for patients with metastatic disease. Despite intensive chemotherapy, metastasis of osteosarcoma occurs in ~20% of cases. Proving there is an urgent need for identifying prognostic markers to facilitate risk stratification. The Wnt/beta-catenin (beta-cat) pathway is dysregulated in osteosarcoma and its role in osteosarcoma progression remains unknown. We investigated the role of the Wnt/beta-cat pathway, specifically the transcriptionally active form of beta-cat, active beta-catenin (ABC), in osteosarcoma progression.

Methods: Using a pEGFP-beta-cat fusion construct plasmid, we carried out site directed mutagenesis to mimic endogenous ABC [GeneArt, Invitrogen]. The pEGFP-ABC and pEGFP-beta-cat constructs were then transfected into SaOS2 cells and subject to Western Blot analysis and immunofluorescence. We also carried out immunohistochemical analysis on 30 OS patients samples to determine whether nuclear ABC levels were correlated to “aggressive” disease.

Results: Initial observations indicated that endogenous nuclear ABC levels, but not beta-cat, increase with osteosarcoma progression. Our pE GFP-ABC/beta cat plasmid constructs showed similar activity to that of the endogenous ABC and beta-cat: endogenous and pEGFP-ABC were both seen in the nucleus while endogenous and pEGFP-beta-cat remained membrane bound. The transfected SaOS2 cells were visualized with immunofluorescence and both the plasmids pEGFP-ABC/beta-cat and endogenous ABC and beta-cat levels were measured with Western Blot analysis.

We analyzed whether nuclear ABC levels were correlated to “aggressive” disease, as determined by metastasis at diagnosis or resection. We observed that a significantly greater number of patients with metastatic disease at diagnosis or at the time of resection showed high nuclear levels of ABC. This strong correlation between high nuclear ABC levels and metastatic disease supports the hypothesis that ABC may be a marker of “aggressiveness” as defined by metastatic potential.
Phenotypic plasticity affords cancer cells with the ability to metastasize and resist therapies, leading to reduced survival in patients. Dynamic regulation of gene expression can be mediated through the epigenome, which is essential for establishing cell fate both in contexts of developmental biology and disease. A tumor micro-environmental factor that can alter the epigenome and transcriptome, as well as lead to an induction of plasticity is hypoxia, a condition of low oxygen.

Here we apply a combination of imaging and high through-put sequencing methods to address how the epigenomes of breast cancer cells respond to hypoxia by examining the alterations in histone modifications in concert with the resulting transcriptional response. T47D and MDA-MB-231 breast cancer cell lines, epithelial and mesenchymal-like respectively, were used. Both electron spectroscopic imaging and immuno-fluorescence imaging reveal extensive, global alterations in the epigenome in response to hypoxia (0.5% O2, 48 hours), exemplified by an enrichment of heterochromatin at the nuclear periphery. Furthermore, at epithelial-to-mesenchymal transition markers and stem cell markers, surrogates for measuring cancer cell plasticity, there is an increase in active histone (H3K4me3) marks upstream of the transcriptional start site with concordant transcriptional up-regulation post hypoxia treatment. Interestingly, we note large differences in the response of T47D and MDA-MB-231 cells, the latter displaying stochastic phenotypic heterogeneity under hypoxia as observed by large variability between the transcriptomes of its three biological replicates.

Taken together, our findings indicate that global epigenetic reprogramming accompanies the hypoxic response, and further suggests the association of epigenetic alterations with the induction of plasticity. Additionally, responses to hypoxia appear to be highly context dependent and heterogenous. Elucidating the nuanced cellular response to hypoxia and the role of the epigenome in this response will better our understanding of hypoxia induced cancer cell plasticity.
Breast cancer cells acquire resistance to Adavosertib through Myt1 upregulation

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Adavosertib is a small molecule Wee1 kinase inhibitor that is currently being tested in the clinic both as a monotherapy and in combination with genotoxic agents. Preclinical and clinical studies have reported that a subset of cancers exhibit intrinsic resistance to Adavosertib. Moreover, cancer cell lines and tumours that are initially sensitive to Adavosertib acquire resistance during treatment course. We used HeLa and a panel of breast cancer cell lines to investigate intrinsic and acquired resistance to Adavosertib. We found that cancer cells range in sensitivity to Adavosertib and that sensitivity to Adavosertib strongly correlates with the levels of the Wee1-related kinase Myt1, a protein that is overexpressed in breast cancer patient tissue. HeLa and MDA-MB-231 cells exhibited high sensitivity to Adavosertib and have low levels of Myt1. However, 30 days of continuous exposure to Adavosertib induces Myt1 upregulation and resistance, a finding that was also observed in animal models.

Wee1 and Myt1 exhibit functionally redundant roles in the inhibition of Cdk1/cyclin B, the key enzymatic complex required for mitosis. Ectopic activation of Cdk1 induces aberrant mitosis and cell death by mitotic catastrophe. Similarly, Adavosertib treatment induces mitotic catastrophe in a subset of breast cancer cells. Cells that are sensitive to the Wee1 inhibitor, exhibit high in vitro Cdk1 activity in the presence of Adavosertib leading to premature mitotic entry from S phase and chromosome fragmentation. As a result, Adavosertib sensitive cells treated with the Wee1 inhibitor arrest in mitosis and die. In contrast, cancer cells that exhibit intrinsic or acquired resistance to Wee1 inhibition exhibit low in vitro Cdk1 activity in the presence of Adavosertib and exhibit normal mitotic timing and progression. Myt1 knockdown by siRNA restores cell sensitivity to Adavosertib. Our data demonstrates that Myt1 upregulation is a mechanism by which cancer cells acquire resistance to Adavosertib.
BAD phosphorylation facilitates mRNA translation during epithelial cell migration in the developing mammary gland

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Postnatal mammary gland development is dependent on tightly controlled cycles of proliferation, migration, differentiation and apoptosis. Identification of genes driving this normal process is central to understanding signaling pathways that are often dysregulated in cancer. We have previously shown the BH3-only protein BAD is a prognostic marker for survival of breast cancer patients. While this suggests that BAD regulates tissue homeostasis, it is not known whether BAD has a physiological role in the mammary gland. BAD is extensively regulated through coordinated phosphorylation of three key serine(S) residues S112, 136 and 155. Using a mouse genetic model wherein the three residues are replaced by alanine (3SA), we characterized different stages of postnatal mammary gland development. BAD 3SA mutant animals showed pubertal-specific delay in ductal elongation and decreased primary branching of the mammary epithelial tree. Transplant of mutant epithelium into cleared wild-type mammary fat pads demonstrated that this effect was derived from the epithelial compartment. Ex vivo 3D organotypic cultures of purified mouse mammary epithelium and human mammary epithelial MCF10A cells showed delayed branching and tubulogenesis, confirming the cell autonomous-effect of BAD phosphorylation. MS screen identified differences in actin-binding and focal adhesion components suggesting defects in cell migration. Additionally, RPPA screen identified deficient phosphorylation of the mRNA translational regulator, 4E-BP1. This is intriguing because localized translation in subcellular protrusions has been shown to drive focal adhesion maturation and cell motility in vitro. Consistent with this, BAD phosphomutant 3D organoids generated cell protrusions with decreased mRNA translation and diminished levels of cell migration effectors, actin and paxillin. Importantly, these nascent protrusions were unstable, and cells displayed diminished migration. Our findings demonstrate that localized translation is critical for cell migration during mammary gland development, identify BAD as a previously unknown regulator of this process and suggest novel pathways to explore in breast cancer pathogenesis.
Fatemeh Mashayekhi

Oncology

Dissecting the Role of polynucleotide kinase/phosphatase (PNKP) in replication fork damage

Fatemeh Mashayekhi, Ismail H. Ismail*

DNA is constantly challenged by DNA damage either spontaneously induced during cellular metabolism or generated by exogenous DNA damaging agents. During DNA replication, DNA lesions can cause the stalling or collapse of DNA replication forks. Following replication fork damage, activation of the DNA damage signal transduction network promotes the restart of replication forks thereby preserving genomic integrity. Stressed forks can be faithfully repaired and restarted using homologous recombination (HR) repair pathway. Recently, using iPOND (isolation of proteins on nascent DNA) combined with mass spectrometry, it was found that the bifunctional polynucleotide kinase/phosphatase (PNKP) is enriched on newly replicated DNA. (PNKP) plays role in non-homologous end joining (NHEJ) DNA repair pathway. Recently, it has been reported that NHEJ machinery protects DNA replication fork. But it is not clear whether or not PNKP, similar to other NHEJ proteins, involves in DNA replication fork protection. In this project, I will provide evidence for a role of PNKP in maintaining genomic integrity after replication fork damage.
Caloric Restriction and Exercise protection from Anthracycline Toxic Effects (CREATE): Preliminary effects on secondary cardiovascular outcomes

Brenna C. Mattiello, Richard B. Thompson, Carla M. Prado, Ian D. Paterson, John R. Mackey, Kerry S. Courneya, Edith Pituskin, Justin Grenier, Amy A. Kirkham

Anthracycline chemotherapy is an effective treatment for breast cancer but is also associated with damage to the myocardium. The purpose of the CREATE study was to determine if short-term lifestyle interventions could attenuate markers of cardiotoxicity in women with breast cancer receiving anthracycline chemotherapy. Forty-eight women (age: 54+/−10; BMI: 28+/−6 kg/m2) were randomly assigned to: 1) 30min of 70-75% heart rate reserve aerobic exercise (EX, n=15) 24h prior to each treatment; 2) caloric restriction (CR, n=16) (food provided for 50% of daily measured caloric requirements) for 48h prior to each treatment; or 3) usual care (UC, n=17). Circulating NT-proBNP, an acute biomarker of cardiotoxicity, was collected before and 24h after the first treatment. Myocardial fibrosis (MOLLI native T1 mapping) and aortic distensibility were assessed by 3T MRI before the first (baseline) and 3wks after the last treatment (follow-up). Adherence was >90% for both interventions. After treatment 1, NT-proBNP was elevated in all groups, but was attenuated in CR (mean+/−SE, 70+/−13 to 219+/−30pg/mL) compared to UC and EX (67+/−12 to 317+/−38 and 85+/−14 to 357+/−54pg/mL, p<0.05). At follow-up, T1 times were increased (indicating myocardial fibrosis) in EX and UC (1257+/−8 to 1280+/−8 and 1240+/−8 to 1263+/−8ms, p<0.01), but were unchanged in CR relative to baseline (1259+/−10 to 1267+/−10ms). From baseline to follow up aortic distensibility did not change in any group. Two days of CR prior to anthracycline treatments appears to attenuates acute circulating and cumulative MRI markers of cardiotoxicity. These preliminary analyses indicate that an acute exercise session did not alter these markers. Forthcoming analyses include assessing the intervention effects on resting and exercise cardiac function and skeletal muscle toxicity. (Funding by Susan G. Komen)
Isaac Menghisteab

*Oncology*

**Macrophage MMP are activated colorectal cancer cell line small extracellular vesicles**

*Isaac Menghisteab, Yulin Xi, Hamda Memon, Courtney Mowat, Allison McNamara, Kristi Baker*

We hypothesize that colorectal cancer small extracellular vesicles induce a pre-metastatic niche in the liver through pre-metastatic activation of Kupffer cells. Kupffer cells are liver macrophages exposed to portal vein blood, which flows from the large-intestine to the liver. We measured the activity of matrix-metalloproteinase in cells treated with purified colorectal cancer small-extracellular vesicles and with cancer cell lines cell culture media depleted of small-extracellular vesicles by ultracentrifugation and chemical inhibition. We are also developing in vivo methods to detect small extracellular vesicle uptake and the enhancement of metastasis establishment by conditioning of in vivo systems with purified cancer cell line small extracellular vesicles prior to the introduction of metastasis forming cells. Our results in the RAW264.7 cell line indicate that cancer cell line small-extracellular vesicles do indeed activate MMP activity. By mass spectrometry of cancer cell lines small extracellular vesicles we have identified a potential mechanism by which matrix-metalloproteinases are activated and are investigating this. Furthermore, we have successfully applied the in vivo liver metastasis model. Future experiments include the induction of metastasis after pre-conditioning of mice with cancer cell lines small extracellular vesicles.
Mahsa Mohseni

MEDICINE

Combination of siRNA and Chemotherapeutic Agents in Acute Leukemic Cells

Mahsa. Mohseni, Cezary. Kucharski, Remant Bahadur. KC, Hasan. Uludag, Joseph M. Brandwein

Introduction:
Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children, accounting for 29% of all pediatric cancers 1. Small interfering RNA (siRNA) mediated silencing of oncogenes in combination with the most common chemotherapeutics used for ALL could improve treatment outcomes of current therapies 2, 3, 4, 5. However, an effective siRNA therapy requires efficient delivery systems since polynucleotides are highly unstable in serum, and their anionic nature prevents them from traversing cellular membranes 5. Among molecular targets for acute leukemia, transcription factors including Signal Transducer and Activator of Transcription (STAT) protein members are highly important, since they activate expression of oncogenes leading to aberrant cancer cell proliferation 4. In hematological malignancies, STAT5 downregulation can decrease leukemia cell proliferation 3, 4, 5. In this study, we evaluated therapeutic role of STAT5 inhibition by polymeric siRNA delivery systems in combination with drugs in ALL cell lines.

Methods:
Acute lymphocytic RS4; 11 and SUP-B15 leukemia cells were used. Lipid-modified low molecular weight polyethyleneimine (PEI) polymers were used as siRNA carriers. Doxorubicine, dexamethasone and vincristine were used as chemotherapeutics. Cell proliferation was assessed by MTT assay, Cellular uptake by Flow Cytometry and STAT5 gene knockdown by RT-qPCR.

Results:
Specific lipid substituted 1.2 and 2 kDa PEIs (1.2PEI and 2PEI) displayed excellent complexation properties with siRNAs to form nanoparticles and gave high siRNA uptake. STAT5 gene expression was downregulated (60-90%) in SUP-B15 and (32%) in RS4; 11 cells using 1.2PEI and 2PEI-lipid polymers. In addition, siRNA complexes in combination with vincristine (10 nM) induced a significant growth inhibition in RS4;11 cells.

Conclusions:
We demonstrated effective STAT5 siRNA delivery by polymeric nanoparticles, accompanied by marked STAT5 gene inhibition. Cell growth was reduced significantly by drug-siRNA combinations. Further experiments will be directed at evaluating STAT5 protein silencing and exploring effect of STAT5 downregulation on ALL patient samples.
Purpose: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcriptional factor, and regulates the expression of various genes. It is well established to play a promoting role in the initiation, promotion, progression, invasion, and metastasis of cancer cells. The full-length AhR encompasses various domains, including bHLH, PAS A, PASB & transactivation domain. The PAS B domain plays a key role in regulating the activity of AhR by interacting with either AhR agonists/antagonists through its ligand binding domain (LBD). Here we focus on using computational modelling to study the structure of the PAS B region and understand its LBD.

Methods: As the crystal structure of the PAS B domain is not available, we used different homology modelling algorithms to build a 3-dimensional (3-D) structure for this region. Our building process involved several steps, including: template identification, target-template alignment, model building, and model validation. Results: The crystal structures of human hypoxia inducible factor (HIF-2α) (PDB code: 5TBM), and (HIF-1α) (PDB code: 4H6J) were used as templates to build different models for the PAS B domain, and have a sequence similarity to PSA B of ~27% and 31%, respectively. Seventeen different PSA B models were built. Fifteen models were constructed based on the two templates using Swiss model, MOE, i-TASSER, and phyer2 software packages. The other two models were generated using an ab initio modeling algorithm as implemented in i-TASSER and phyer2. The models were validated using several tools, and two models showed acceptable scores. Conclusions: The PSA B domain of the AhR contains LBD. Two models showed acceptable 3-D parameters and will be used to understand how the PSA B domain regulates the function of AhR. This will lead to the development of new therapies for the treatment cancer.
Background: Studies investigating energy requirements in cancer are inconsistent and requirements may be impacted by cancer therapy. Adequate energy intake is essential to sustain body weight and muscle mass during cancer disease trajectory. Objective: The aim of this study was to investigate therapy’s impacts on energy expenditure and body composition in patients with stage II-IV colorectal cancer. Methods: This prospective cohort study measured several indices of nutritional status before and after chemotherapy and/or radiation treatment. Resting energy expenditure (REE) was measured using a whole-body calorimetry unit and body composition by bioelectrical impedance analysis (Bodystat Quadscan 4000). Body mass, calf circumference, and Patient-Generated Subjective Global Assessment (PGSGA) Short Form were also evaluated. Effect sizes were calculated using Cohen’s d formula and classified as small (d=0.2), medium (d=0.5), and large (d=0.8). Results: Five participants (n=4 men, 56±8 years of age) were enrolled in this pilot study. One participant started an exercise program during cancer treatment and was analyzed separately due to discrepant changes. In participants not involved in the exercise program, body mass index (26.1±5.2 vs 25.8±4.5 kg/m², d=0.27), calf circumference (36.6±3.5 vs 36.0±2.8 cm, d=0.72), PGSGA (6.5±5.0 vs 3.8±2.4, d=0.83), phase angle (5.7±1.0 vs 5.5±0.7, d=0.47), and lean mass (58.0±16.3 vs 57.1±15.5 kg, d=0.41) decreased during cancer treatment. REE also decreased (1842±351 vs 1706±405 kcal/day, d=1.04). No effect was observed for fat mass (21.4±7.4 vs 21.3±6.0 kg, d=0.07). The participant enrolled in the exercise program increased BMI (20.6 vs 23.4 kg/m²), lean mass (41.2 vs 49.0 kg), calf circumference (31.9 vs 34.0 cm), and REE (1603 vs 1951 kcal). Conclusion: Cancer therapy impacts REE likely related to lean mass changes. Findings from the study support developing of larger trials exploring changes in energy metabolism during cancer disease trajectory.
Colorectal cancer (CRC) is the second most common type of cancer in Canada, with the third highest mortality in women and second highest in men. 15% of CRCs fall into the microsatellite instable (MSI) subtype, characterized by genetic instability and stronger immunogenicity, leading to better prognosis than the more common microsatellite stable (MSS) subtype. In non-cancer cells, pattern recognition receptors (PRRs) respond to damage associated patterns to activate the innate immune system. Specifically, the cGAS/STING pathway recognizes foreign DNA in the cytosol leading to production of type I interferons. In cancer cells, cGAS/STING are also capable of recognizing self cytosolic DNA generated through DNA damage (cyDNA). I aim to characterize the cyDNA present in MSI vs MSS CRC cells and understand how this differentially affects anti-tumor immunity. To do this I will sequence cyDNA in MC38 CRC cells where we knocked out Mlh1 (MSI), the PolE gene associated with hypermutability or Kras or Rad51 (MSS), and determine the resulting effect on PRR signalling. We will also treat cells with the commonly used DNA damaging treatments irradiation and 5-fluorouracil (5-FU) since MSI CRCs are known to respond differently. Preliminary cyDNA stimulations indicate differential cGAS/STING pathway activation between the CRC subtypes at baseline. We have also observed a dose dependent effect for cyDNA following irradiation that leads to increased concentrations of cyDNA in as early as 30 minutes. During stimulations this irradiated cyDNA from the MSI, PolE, and Rad51 lines leads to a stronger response. Preliminary stimulations with 5-FU treated cyDNA also suggest stronger cGAS/STING activation. With immune recognition of CRC being crucial to treatment efficacy, this research will shed light on the relationship between genetic damage in cancer cells and PRR sensing and lead to a method of characterization of tumor types relative to immune activation.
Ashley Newbigging

*Laboratory Medicine and Pathology*

**Enzyme-free and wash-free technique for cell surface imaging**

*Ashley M. Newbigging, Hongquan Zhang, X. Chris Le*

HER2 is a transmembrane protein found on HER2+ breast cancer cell surfaces. These cancers are known to be more aggressive and have a higher chance of recurrence. An effective treatment, trastuzumab, specifically targets the overexpressed HER2 proteins. Therefore, it is necessary to determine the HER2 status of breast cancer patients to determine their eligibility for treatment. The method to determine HER2 status of patients is through immunohistochemistry. Immunostaining methods including immunohistochemistry are time consuming and laborious as they require multiple wash steps and the use of enzymes to confer amplified signals, both of which can increase the turnaround time for these patients. We have developed a novel technique for cell imaging without requiring wash steps or enzymes using the rational design of nucleic acids. Our technique uses two DNA probes and four DNA hairpins. The hairpins are complementary to each other, but they cannot react because they are locked within the hairpin conformation. The two probes consist of DNA oligonucleotides conjugated to anti-HER2 antibodies. The DNA oligonucleotides can only bind to each other when the antibodies bind to adjacent HER2 molecules or adjacent epitopes on one HER2 molecule. When bound together, the probes initiate binding to each of the hairpins subsequently, resulting in the formation of a long, nicked polymer. The formation of the polymer further creates a complementary sequence that is detected using fluorophores. We applied our technique to SK-BR-3 (HER2+) and MDA-MB-231 (HER2-) cell lines. We achieved the specific detection of HER2+ live cells in less than 45 min without requiring any enzymes or wash steps using our technically simple procedure.
Docosahexaenoic acid (DHA) enhances the action of relevant cytotoxic drugs and reduces the growth of immortalized breast cancer cell lines in vitro and in vivo. We sought to confirm this in a more clinically translatable heterogeneous model, using BC patient derived xenografts (PDXs). Female NSG mice bearing triple negative breast cancer PDX tumors (100 mm³) were randomized to one of two nutritionally adequate high fat diets (20% w/w ± DHA). Treatment paradigms included a) control (0% dietary DHA); b) control+docetaxel (TXT 5mg/kg; intraperitoneal) and c) 4% w/w of fat DHA+TXT (n=7/group). After 6 weeks of chemotherapy, tumors were excised, weighed and the phospholipid fatty acid composition determined. Feeding 4% DHA decreased tumor growth by 43% and 34% compared to control or control+TXT, respectively (P<0.05) and increased tumor phospholipid DHA compared to control+TXT (5.7 ± 0.3% vs 3.8±0.2%, P<0.05). We further sought to determine the mechanisms through which DHA elicits this anti-tumor effect. An increase in necrotic tissue was observed in immunohistochemical haematoxylin and eosin (H and E) staining of the DHA+TXT tumors compared to both control diet groups. Protein analysis confirmed a higher expression of proteins involved in necroptosis: Ripk1, Ripk3 and MLKL, coinciding with a lower NFκB protein expression in tumors from the DHA+TXT group (P <0.05). This study suggests, that feeding a diet supplemented with DHA facilitates the anti-cancer effect of TXT on breast cancer PDXs, at least in part, through increased necroptosis. (supported by CIHR)
The transcription factor, p53, is a potent tumor suppressor protein. It plays a major role in the regulation of the cellular machinery in response to a variety of oncogenic stresses. Hence, the inactivation of p53 is an efficient strategy adopted by cancer cells to promote their survival and progression. In fact, p53 is the most mutated protein in cancer.

Plakoglobin, which is a tumor suppressor catenin, has dual signaling and adhesion functions in cells. One mechanism through which plakoglobin confers its tumor suppressor ability is by interacting with and regulating the expression of p53 target genes. Our collaborators have further shown that plakoglobin interacts directly with mutant p53 to restore its wild-type conformation.

We used in silico modeling to predict the structure of R175H mutant p53-plakoglobin complex on an atomistic scale. Co-threading was first used to predict the initial relative positions of the complex atoms. To sample the conformational space efficiently, we simulated the system using accelerated molecular dynamics for 500 ns. These simulations rely on reducing energy barriers to enhance conformational space sampling. Clustering was used to group similar conformations of the complex together. MMGBSA calculations were used to estimate the average binding energy of p53 to plakoglobin for each cluster. Complex residues, which contributed the most to the lowest energy complex structures were identified for testing using in vitro site-directed mutagenesis experiments. Our next aim is to find small molecules that could mimic the rescuing effect of endogenous plakoglobin on mutant p53 for use in cancer treatment.
BIK drives an aggressive breast cancer phenotype through sublethal apoptosis and predicts poor prognosis of ER-positive breast cancer

Vrajesh Pandya, John Maringa Githaka, Namrata Patel, Richard Veldhoen, Judith Hugh, Sambasivarao Damaraju, Todd McMullen, John Mackey, Ing Swie Goping

Apoptosis is fundamental to normal animal development and is the target for many anti-cancer therapies. Recent studies have explored the consequences of “failed apoptosis” where the apoptotic program is initiated but does not go to completion and does not cause cell death. Nevertheless, this failed apoptosis induces DNA double-strand breaks generating mutations that facilitate tumorigenesis. Whether failed apoptosis is relevant to clinical disease is unknown.

BCL-2 interacting killer (BIK) is a stress-induced BH3-only protein that stimulates apoptosis in response to hormone and growth factor deprivation, hypoxia and genomic stress. It was unclear whether BIK promotes or suppresses tumor survival within the context of breast cancer. We investigated this and show that BIK induces failed apoptosis with limited caspase activation and genomic damage in the absence of extensive cell death. Surviving cells acquire aggressive phenotypes characterized by enrichment of cancer stem-like cells, increased motility and increased clonogenic survival. Furthermore, by examining six independent cohorts of patients (total n=969), we discovered that high BIK mRNA and protein levels predicted clinical relapse of Estrogen receptor (ER) positive cancers, which account for almost 70% of all breast cancers diagnosed but had no predictive value for hormone receptor-negative (triple-negative) patients. Thus, this study identifies BIK as a biomarker for tumor recurrence of ER-positive patients and provides a potential mechanism whereby failed apoptosis contributes to cancer aggression.
Despite major advances in treatment for early stage breast cancer, metastatic breast cancer (MBC) remains incurable due to resistance to most available treatments. Furthermore, toxicity with metastatic regimens can be quite high. Both diet and exercise have been used to attenuate treatment toxicity, but their potential to enhance chemotherapy efficacy and survival has not been studied in humans. Aerobic exercise substantially increases tumor blood flow and oxygen delivery, suggesting that exercise during chemotherapy could increase delivery to the tumor and reduce hypoxia. Acute caloric restriction also appears as an effective strategy to inhibit tumor growth and enhance chemotherapy efficacy. The DREAM study is a phase II, two-arm, single blind, RCT. Fifty women with MBC receiving first or second line IV chemotherapy will be randomly assigned to the combined aerobic exercise and caloric restriction intervention or to usual care. Exercise will consist of a supervised recumbent cycling session performed concurrent to each chemotherapy infusion for 45mins. A freshly prepared diet consisting of low-carbohydrate meals with a caloric content of 50% of energy requirements will be provided to intervention group participants for the 72h prior to each chemotherapy. The primary aim of this study is to evaluate the efficacy of combining the short-term exercise and diet intervention for up to six chemotherapy treatments on tumor size (CT scan). Secondary outcomes include a MRI markers of tumor response, quantitative treatment toxicity (MRI of cardiac function, skeletal muscle and liver), patient-reported symptoms and quality of life. Progression-free and overall survival are exploratory outcomes. Recruitment began in July 2019 and is expected to take 24 months. This intervention is easily accessible, low risk, low cost, and associated with other known benefits. Our approach has the potential to improve tumor response to chemotherapy and reduce side effects; therefore, increasing progression-free and overall survival.
Poster 67

Desmond Pink

Oncology

Translating a MicroFlow Assay from the Lab to the Clinic: A Real-World Experience in Progress

Desmond Pink Nanostics, Robert Paproski, Nanostics Catalina Vasquez, Nanostics Michael Wong, Nanostics Renjith Pillai, Nanostics Diana Pham, Nanostics Rebecca Hiebert, Nanostics Leanne Stifanyk, DynaLIFE Medical Labs Sylvia Koch, DynaLIFE Medical Labs John Lewis, Nanostics

Nanostics, in partnership with a major Canadian medical laboratory called DynaLIFE, is translating the microflow assay called ClarityDX Prostate designed for identification of aggressive prostate cancer, to the clinic. Building upon a previous prospective analysis using frozen samples, we are validating our test in fresh blood samples from Albertan men suspected of prostate cancer. Our current data provide insight into some of the logistical issues and realities of translating an EV microflow cytometry test to an effective clinical workflow.

To ensure the highest rigor, translating our EV test to a clinical microflow pathway has involved discussions with regulatory consultants, statisticians, clinical flow cytometry operators, feasibility experts, clinical coordinators, and physicians. Initial engagement with physicians early in the process affirmed the clinical unmet need and that it’s great to have a new test but it must have the potential to significantly improve clinical outcomes. The regulatory consultants and statisticians provided a reality check as to the amount of testing that needs to be conducted to translate a test to the clinic. Since the clinical lab and the academic lab workflows are very different, discussions with clinical flow cytometry operators provided an unbiased assessment of how our SOPs and workflow would translate. Keeping a test as streamlined as possible improves overall feasibility because each additional step adds time, complexity, variability and cost. Clinical coordinators navigate ethics to streamline SOPs for patient consultation and sample collection. The SOPs for sample collection, shipping and processing were written and validated so that each procedural step could be performed by test-naive operators following a brief training period.

Adapting our test to a valid clinical workflow is a significant and challenging undertaking. In this discussion we outline our experiences as we translate our test from the bench to a real-world clinical workflow.
Multiple myeloma (MM) is a blood cancer where non-functional abnormal plasma cells are produced in large amounts that crowd out healthy plasma cells in the bone marrow and interfere with the production of other blood cells. Despite current advances in MM treatment, it remains an incurable disease and new treatment options are urgently needed.

The genomic instability in MM cells have been attributed to replication stress (RS). Replication stress can be defined as the slowing or stalling of replication fork or DNA synthesis. A hallmark of replication stress is generation of stretches of single stranded DNA (ssDNA) which are then bound by replication proteins A (RPA). ssDNA-RPA recruits RS proteins including the kinase ATM and Rad3 related (ATR) which phosphorylates downstream proteins and allows cell survival. Specific readouts of RS include RPA phosphorylation at threonine 21, serine 4/8 and serine 33. In this project we have identified two MM cell lines that have defective RS response shown by reduced RPA phosphorylation in response to ATR inhibitor treatment under replication stress. We have also confirmed that this phenotype is not due to double strand break or difference in cell cycle profile. We therefore hypothesize that this difference in replication stress response in MM cell lines is due to differential expression of oncogenes among the cell lines. To test this hypothesis we will check expression pattern of oncogenes in MM cell lines under replication stress condition. We will also knock down the oncogenes to see if we can create normal RS response in the defective cell lines.

The goal of this project is to elucidate the mechanism of RS in MM cells and identify potential ways in which this knowledge could be used for the treatment and diagnosis of MM.
Solid tumours are often poorly oxygenated; this phenomenon is clinically known as tumour hypoxia. The presence of hypoxia in tumours results in resistance to standard chemotherapeutic and radiotherapy, and thus may contribute to treatment failure and relapse. Targeting hypoxia, therefore, is vital for the therapeutic management of solid malignancies. 2-Nitroimidazoles (2-NI) are particularly interesting for hypoxia-directed therapy because of their unique bioreduction potential. Reduced 2-NI compounds get selectively entrapped in hypoxic cells by forming drug-protein adducts. As a result, radiolabeled 2-NIs are widely used as radiodiagnostic tools to monitor tumour hypoxia. Clinically established examples include our compounds of interest; 123I-iodoazomycin arabinoside (123I-IAZA) and 18F-fluoroazomycin arabinoside (18F-FAZA). In this study, we are exploring the therapeutic potential of non-radiolabelled analogues of 2-NI radiotracers, IAZA and FAZA, in a head and neck tumour model. We report that both compounds are selectively toxic to hypoxic cells with a profound effect on cell proliferation. We hypothesize that hypoxic sensitization by 2-NI compounds is achieved partly through their interaction with critical cellular proteins. To identify the potential interaction partners of our drugs, we have developed a clickable 2-NI which allows us to pull down drug-protein adducts using a biotin alkyne. Using LC-MS/MS, we have identified multiple potential hits and are currently working on the functional consequences of drug-protein interaction. The clickable 2-NI also offers an easier, faster and cheaper alternative to Pimonidazole immunohistochemistry for hypoxia mapping on cells and tissues. In addition, we have also explored the radiosensitization potential of IAZA and FAZA, and have observed an additive effect in hypoxic cells treated with drug and radiation. We have generated the toxicity profile of single i.p. administered IAZA, and have performed an in vivo radiosensitization study in a xenograft mouse model. Although single IAZA administration did not provide survival advantage, we observed an initial tumour growth delay in IAZA injected mice; combining IAZA with a single radiation dose however did not provide additional benefit compared to radiation treatment alone.
Mitotic catastrophe is a common mode of tumour cell death associated with an aberrant mitosis. Because most cancer cells have one or more defective cell-cycle checkpoints, exposure to genotoxic agents, and/or small molecular inhibitors of checkpoint effector kinases, can force cells into mitosis with damaged or under-replicated DNA. Centromeric DNA is replicated late relative to other regions of the chromosomes which makes centromeric DNA prone to breakage during premature mitosis. This process is referred to as centromere fragmentation.

In this work, we present an automated system for segmentation of centromere fragmentation cells. The main challenge to develop an automated system is posed by the fact that cells grow in clumps which make identification of single cell problematic. Thus, we developed a 2-phase system for automating the segmentation process. The first phase was aimed at segmenting individual cells from clumps. We proposed a new variation of Chan & Vese based level set method, and added two new terms in the energy functional of the traditional Chan & Vese model namely Weighted Local Repelling term (WLrt) and Weighted Gradient term (WGt). The first term namely WLrt was responsible to generate a huge repelling force as soon as contours of different cells touched each other. The second term, WGt, was responsible to enhance gradients between touching cells. The second phase was designed to identify cells with centromere fragmentation. We proposed a new algorithm namely SBaC (Shape Based analysis of each Chromosome) which extracts features like circularity, major-axis length, minor-axis length, area and eccentricity from each chromosome to distinguish normal cells from cells having centromere fragmentation. Further, the performance of both WLrt- WGt-LSM and SBaC was evaluated using various evaluation metrics like Dice, Volume Error, Intersection over union, Area under ROC curve, Precision, Recall, F1-score. The proposed system could identify centromere fragmentation cells robustly.
Introduction: 6-Mercaptopurine (6-MP) is a nucleobase analog drug used in the maintenance treatment phase of acute lymphoblastic leukemia (ALL). Our lab has established that transfection of cells with SLC43A3, which encodes equilibrative nucleobase transporter 1 (ENBT1), increases 6-MP influx and cytotoxicity in a heterologous transfection model. SLC43A3 is known to be expressed in leukemia cells, but its relationship to 6-MP therapeutic activity has not been defined. We hypothesize that the level of SLC43A3 expression in leukemia cells relates directly to the degree of 6-MP uptake and cytotoxicity.

Methods: A panel of leukemia cell lines was assessed for SLC43A3 expression, ENBT1 function, and sensitivity to 6-MP cytotoxicity. SLC43A3 transcript was determined using RT-qPCR. 6-MP cytotoxicity was assessed using the MTT assay. ENBT1 function was evaluated by measuring the 2 sec uptake of [14C]6-MP by these cells. SLC43A3 knockdown was achieved using the SMARTvector Lentiviral shRNAi inducible vector.

Results: SLC43A3/ENBT1 expression and function varied widely across leukemia cell lines. We found significant correlations between SLC43A3 expression, 6-MP uptake, and toxicity to 6-MP. Furthermore, knockdown of SLC43A3 with shRNAi in the RS4:11 cell line led to a significant decrease in 6-MP uptake (3-fold) and cytotoxicity (Log EC50: ALL-1 = -6.45 ± 0.11; ALL-1_shRNAi = -4.242 ± 0.14)

Conclusions: Changes in expression and activity levels of SLC43A3/ENBT1 lead to concurrent changes in 6-MP cytotoxicity. These data suggest that ENBT1 is the primary transporter for 6-MP in leukemia cell lines and SLC43A3 may be a genetic biomarker for 6-MP dosage efficacy in ALL.
Sams Sadat

Pharmacy and Pharmaceutical Sciences

A new synthetically lethal nanomedicine for colorectal cancer therapy.

Sams M. A. Sadat, Igor Paiva, Marco Paladino, Feridoun Karimi-Busher, Dennis Hall, Michael Weinfeld, Afsaneh Lavasanifar

Purpose: Phosphatase and TEnsin homolog (PTEN) is a tumor-suppressor gene that is lost in up to 75% of aggressive colorectal cancer (CRC). The co-depletion of PTEN and a DNA repair enzyme known as polynucleotide kinase phosphatase (PNKP), has been identified to lead to synthetic lethality in several cancer cell lines including CRC cells. Our aim was 1) to develop novel PNKP inhibitors and their nanoparticle (NP) formulations for tumor enhanced delivery; and 2) to investigate the anticancer activity of PNKP inhibitor as free and NP formulation in wild-type (PTEN+/+) and PTEN-deficient (PTEN-/−) CRC xenograft in mice.

Methods: A83B4C63 was either encapsulated in methoxy poly(ethylene oxide)-b-poly(α-benzyl carboxylate-ε-caprolactone) (PEO-b-PBCL) NPs or solubilized with the aid of Cremophor EL: Ethanol (CE). The biodistribution of A83B4C63 for both formulations was determined in HCT116 (PTEN positive and negative) tumor xenograft bearing animals (n=4) 24 h after 3 every other day intravenous (IV) injections of 25 mg/kg. Tumor tissues were also immunostained with Ki-67 antibody. The anticancer activity of both formulations was determined in both xenografts in NIH-III nude mice (n = 8) following the same dosing schedule repeated after a one week gap for a total of 6 IV injections.

Results: A significantly higher concentration of A83B4C63 was measured in plasma, tumor, and liver when delivered by NPs compared to CE formulation in HCT116/PTEN-/− xenografts. The NPs of A83B4C63 reduced the rate of HCT116/PTEN-/− xenograft growth more efficiently than free drug. This was in contrast to wild-type xenografts, which showed similar growth rates following systemic administration of A83B4C63 in either formulation, formulation excipients (without drug) or 5% dextrose. In vivo anti-proliferative efficacy was observed only in PTEN-deficient tumors-bearing xenograft in mice that received the NPs of A83B4C63.

Conclusion: Delivery of A83B4C63 by PEO-b-PBCL NPs demonstrates a promising new monotherapeutic option in PTEN-deficient CRC.
Oral squamous cell carcinoma (OSCC) is the most prevalent and dangerous form of oral cancer with an average 5-year survival rate of 57%. Surgery and radiation therapy accompanied by chemotherapy are treatment options for OSCC. Platinum(Pt)-based antineoplastic agents, are commonly used via systemic administration. The nephro- and neurotoxicity of these agents following systemic administration, limits their clinical use. Local delivery of such agents to the oral tumor can assist in reducing the toxicities of therapy while providing a higher concentration of the drug at the tumor site, in-comparison to systemic administration.

In this work, we evaluated the potential application of a novel mucoadhesive hydrogel based on poly(acrylic acid) grafted cellulose nanocrystal (CNC-PAA), for local delivery of cisplatin in OSCC. The developed formulations were evaluated for their cytotoxicity against two human OSCC cell lines, HSC-3 and OSC-19. In vitro release of platinum from the hydrogel films was also studied via the dialysis method. Additionally, we described the designed and application of a novel apparatus used to evaluate the ex vivo muco-retentive behavior of the developed hydrogels by measuring the kinetics of gel/platinum wash out from the surface of porcine buccal tissue by simulated saliva fluid.

Our results showed a lower in vitro cytotoxicity for the CNC-PAA formulation of cisplatin compared to the free drug (2-3 fold increase in IC50), owing to a slower drug release from this formulation. Only 25% of incorporated cisplatin was released from CNC-PAA-Pt complexes after 2 hr compared to > 80 % release of free cisplatin, at the same time point. The results from muco-retentive apparatus confirmed the advantage of PAA-grafted-CNC compared to PAA or CNC alone in the formation of muco-retentive hydrogels. The findings demonstrated the potential applicability of the CNC-PAA as a safe and efficient nano-gel carrier for Pt-based chemotherapeutic in oral cancer therapy.
Characterization of novel HEK293 cell lines to assess the regulation of equilibrative nucleoside transporter proteins

Nayiar Shahid, Khanh Hoa Nguyen, Chris Cromwell, Basil P Hubbard, and James Hammond

Background: Equilibrative nucleoside transporters (ENT1, ENT2) mediate the transmembrane flux of endogenous nucleosides along with nucleoside-analogue drugs used in anti-cancer (gemcitabine, cytarabine) and anti-viral (ribavirin, and zidovudine) therapies. An understanding of how ENT function is regulated is critical to controlling/exploiting its impact on therapies using nucleoside analogues.

Objectives: Previous work indicates that ENT1 is regulated by protein kinase C (PKC), protein kinase A (PKA) and casein kinase II (CKII) and may involve interactions between ENT1 and ENT2. To assess underlying mechanisms, we have developed a series of novel Human Embryonic Kidney (HEK293) cell mutants (using CRISPR-cas9) lacking both ENT1 and ENT2 subtypes (HEK293-NTD), or just expressing ENT1 (HEK293-ENT2KO), or just ENT2 (HEK293-ENT1KO). We now report on the characteristics of these cell lines, in terms of ENT expression levels and functional activities.

Methods: ENT1 and ENT2 function was assessed by measuring the initial rates of [3H]2-chloroadenosine uptake (2.5 – 300 µM) and binding of the specific ENT1 probe [3H]NBMPR. Protein levels were assessed by immunoblotting using ENT-specific antibodies. We also confirmed the affinities of a range of known nucleoside/nucleobase substrates for their transporter subtype selectivity.

Results: HEK293-ENT2KO had a similar level of ENT1 as wild-type HEK293 cells (Km-38.7±6.9 µM, Vmax-5.4±0.6 pmol/µl/s) and HEK293-ENT1KO had a similar level of ENT2 (Km-104.7±51.94 µM, Vmax-1.62±0.35 pmol/µl/s) as the wild-type. The isolated transporter subtypes have the expected affinities for a range of nucleoside/nucleobase substrates and known inhibitors.

Conclusion: We will use these models to study posttranslational versus transcriptional regulation of EN Ts and protein interactions/oligomerization in a defined human cell milieu. These investigations will advance our knowledge of pathways that may be exploited to modify cellular function and enhance the therapeutic use of anticancer and antiviral nucleoside analogues.
Tipifarnib and Adavosertib combination treatment synergistically kills breast cancer cells.

Joanne D. Smith, Cody W. Lewis, Gordon K. Chan

The mitotic checkpoint is a failsafe mechanism for the cell to ensure correct chromosome segregation. The evolutionarily conserved kinetochore RZZ complex is an essential component of the mitotic checkpoint. Spindly interacts with the RZZ complex in a farnesylation dependent manner and acts as a dynein adaptor at kinetochores. Spindly knockdown results in prometaphase delay, alignment defects, and loss of dynein kinetochore localization. The knockdown of Spindly phenocopies farnesyl transferase inhibitor (FTI) treatment of cells suggesting that Spindly is the mitotic target of FTIs. FTIs were originally developed to target the RAS oncogene, but have been abandoned as inhibitors of the Ras pathway. Our findings indicate a possibility in repurposing FTIs for cancer treatment, especially in combination treatment strategies.

Wee1 is a kinase that inhibits Cdk1 and when inhibited results in cells undergoing premature mitosis. Wee1 is also required to inhibit Cdk1 at the end of mitosis for mitotic exit and Wee1 inhibition results in mitotic arrest. Adavosertib, a small molecule inhibitor of Wee1 kinase activity, is currently in Phase I/II clinical trials for many cancers. We have found that Adavosertib treatment results in mitotic catastrophe and enhances the sensitivity of breast cancer cells to anti-mitotic agents, such as paclitaxel or farnesyltransferase inhibitors. Our data indicates that the synthetic lethal relationship between Tipifarnib (a FTI) and Adavosertib, both of which are being used in clinical trials for breast cancer individually, is conserved in a subset of breast cancer cells. Preliminary data also indicates that the order in which these 2 compounds are added influences the cell killing observed. The minimum Tipifarnib treatment time and concentration was determined and further experiments are required to confirm these results to understand the mechanism considering this new information. The combination treatment of Tipifarnib and Adavosertib represents a potential new treatment for breast cancers.
Cytotoxic activity of Gaillardia aristata leaf extract on HeLa cells

Sargun Sokhi, Cody W. Lewis, Joanne Smith and Gordon Chan

Cytotoxic activity of Gaillardia aristata leaf extract was examined on HeLa cells. This project is a collaboration with the Prairie to Pharmacy program led by Dr. Roy Golsteyn (University of Lethbridge). This program examines the under-explored prairie plants for anti-tumor activities. Prairie plants have co-evolved with harsh climate and grazing animals and this has led them to produce chemicals to defend themselves. Gaillardia aristata leaf extract was previously shown to have cytotoxic and anti-mitotic activities in initial screenings.

Hypothesis: Gaillardia aristata leaf extract induces a prometaphase like arrest in HeLa cells.

Methods: High Content Imaging system was used to determine mitotic duration. HeLa cells, a human cervical cancer cell line with a competent mitotic checkpoint, were used as a model. Immunofluorescence was used to examine microtubule organization during mitosis.

Results: To determine the most effective concentration of the leaf extract for inducing mitotic arrest, mitotic durations of HeLa cells treated with increasing concentration of leaf extract were examined. 1.5 and 5 ug/mL leaf extract induced the most prolonged mitotic arrest in HeLa cells. Death in mitosis also increased with the increasing concentrations of the leaf extract.

To examine whether the mitotic arrest is due to microtubule perturbation, HeLa cells treated with the leaf extract were examined by immunofluorescence and confocal microscopy. Leaf extract treated cells form normal bipolar mitotic spindles. No disruption of microtubule organization was observed.

Conclusion: G. aristata leaf extract induces a prometaphase like arrest in HeLa cells. G. aristata leaf extract does not inhibit microtubule organization

Future investigation: 1) testing the leaf extract in other cell lines; 2) testing the purified active compound, Pulchelloid A; 3) examine kinetochore-microtubule attachment.
>95% of glioblastoma patients are deceased within 5-years of diagnosis despite aggressive treatment regimens and decades of research–highlighting a need for novel, more effective treatment modalities to combat this malignant brain tumor. Image-guided radiotherapy is a staple for combating glioblastoma by precisely delivering radiation to tumors using cancer imaging techniques. Despite the accuracy image-guided radiotherapy offers, infiltrative tumor-microextensions into normal tissue are still missed and radiation may induce cancer cells to become senescent (i.e. growth-arrested, but still alive), both of which are posited to contribute to cancer recurrence and progression. Therefore, elimination of infiltrative tumor-microextensions and radiation-induced senescent cancer cells may be required for complete eradication of disease. Oncolytic virus therapy harnesses tumor-selective viruses to kill cancer cells while simultaneously jarring the host immune system to attack cancer. Together, combining oncolytic virus therapy with radiation may optimize therapeutic efficacy. Here, we propose to combine our unique mCherry-tagged mutant vaccinia virus (ΔF4LΔJ2R), which exhibits tumor-selectivity due to mutations in key viral nucleotide biosynthesis genes, with image-guided radiotherapy executed using state-of-the-art Small Animal Radiation Research Platform (SARRP) technology. We hypothesize that combining our oncolytic vaccinia virus with image-guided radiotherapy will produce better tumor control than either modality alone, by generating additive or synergistic effects in which image-guided radiotherapy targets the tumor mass while our OV clears tumor-microextensions missed by radiation and/or residual radiation-induced senescent cancer cells.
Yasser Tabana

Pharmacy and Pharmaceutical Sciences

Immunomodulatory effects of Compound-A: a promising treatment to combat cancer

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Purpose: Cancer immunotherapy has emerged as the fourth pillar of cancer treatment along with surgery, radiation, and chemotherapy. Immunotherapeutic approaches utilize components of a patient’s own immune system to selectively target cancer cells. Stimulating the immune system to fight the tumor plays an important role in nearly all aspects of cancer immunotherapy. The objective of our study is based on developing accessible and potent cancer immunotherapy small-molecule drug. Methods: In our study, a heterocyclic compound (Compound-A) was studied for its immunostimulatory effects using peripheral blood mononuclear cells (PBMCs) collected from healthy volunteers. The effects of the compound-A on proliferative capacity and interleukin-2 (IL-2) secretion ability of T cells were determined by carboxyfluorescein succinimidyl ester (CFSE) staining and ELISA, respectively. In addition, microsomal stability of the compound in human liver microsomes and its potential toxicity against PBMCs was determined using CellTiter-Glo® Viability Assay. Finally, the impact of compound A on the regulation of different gens was studied using RNA sequencing approach. Results: Compound-A significantly enhanced immune responses by increased T cells proliferation and IL-2 secretion. Compound-A metabolic stability showed that 59.6 % was remaining after 60 min. No cytotoxicity was shown against PBMCs. A total of 1235 differently expressed genes (DEGs) were identified after treating PBMCs with the compound-A for 12 hours, including 246 upregulated and 989 downregulated genes. Also, a total of 1203 DEGs were identified after 24 hours treatment, including 193 upregulated and 117 downregulated genes. GO and genome pathway analysis showed that these DEGs were enriched in signaling pathways associated with toll like receptor signaling pathway. Conclusions: Our study confirmed the immunostimulatory activities of the Compound-A. Further analyses are required to confirm the molecular pathways underlying activity of this compound as an effective anticancer treatment. A future direction will be to identify and validate the molecular targets responsible for its immunological activities.
Inhibition of autotaxin with GLPG1690 increases the efficacy of radiotherapy and chemotherapy in a mouse model of breast cancer

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Autotaxin catalyzes the formation of lysophosphatidic acid, which stimulates tumor growth and metastasis and decreases the effectiveness of cancer therapies. In breast cancer, autotaxin is secreted mainly by breast adipocytes, especially when stimulated by inflammatory cytokines produced by tumors. In this work, we studied the effects of an ATX inhibitor, GLPG1690, which is in Phase 3 clinical trials for idiopathic pulmonary fibrosis, on responses to radiotherapy and chemotherapy in a syngeneic orthotopic mouse model of breast cancer. Tumors were treated with fractionated external beam irradiation, which was optimized to decrease tumor weight by ~80%. Mice were also dosed twice daily with GLPG1690 or vehicle beginning at one day before the radiation until 4 days after radiation was completed. GLPG1690 combined with irradiation did not decrease tumor growth further compared to radiation alone. However, GLPG1690 decreased the uptake of 3′-deoxy-3′-[18F]-fluorothymidine by tumors and the percentage of Ki67 positive cells. This was also associated with increased cleaved caspase-3 and decreased Bcl-2 levels in these tumors. GLPG1690 decreased irradiation-induced C-C motif chemokine ligand-11 in tumors and levels of interleukin-9, interleukin-12p40, macrophage colony-stimulating factor and interferon g in adipose tissue adjacent to the tumor. In other experiments, mice were treated with doxorubicin every 2 days after the tumors developed. GLPG1690 acted synergistically with doxorubicin to decrease tumor growth and the percentage of Ki67 positive cells. GLPG1690 also increased 4-hydroxynonenal-protein adducts in these tumors. These results indicate that inhibiting ATX provides a promising adjuvant to improve the outcomes of radiotherapy and chemotherapy for breast cancer.
Inorganic arsenic is a toxic element that originates from the earth’s crust. Arsenic can be released into the environment through both human activities such as mining, and natural sources such as volcanic eruptions. One main source of human exposure to arsenic is drinking water obtained from groundwater sources. Exposure to high concentrations of arsenic contributes to illnesses such as skin lesions, cardiovascular disease, neurological diseases, respiratory problems, and cancers. Humans can metabolize ingested inorganic arsenic into less toxic methylated forms. There is significant variability in susceptibility to arsenic toxicity among humans, and this may be because of differences in human metabolism of arsenic. In fact, two people exposed to the same amount of arsenic can show different clinical symptoms. There are numerous factors that can affect an individual’s susceptibility to arsenic toxicity, such as the species of arsenic an individual is exposed to, age, and gene expression for enzymes that metabolize arsenic into less toxic forms. I will test the hypothesis that the profiles of arsenic metabolites in the urine of individuals can serve as biomarkers to estimate their susceptibility to arsenic toxicity. The profile of arsenic metabolites in urine allows for the determination of the ratio of inorganic arsenic to methylated arsenic and the assessment of how efficiently an individual can convert arsenic into less toxic forms. My supervisor and I are collaborators with researchers at the University of Chicago on an epidemiological study in an arsenic-affected population in Bangladesh to determine whether differences in methylation and excretion of arsenic species may correlate to the incidence of arsenic-induced illnesses. My contribution to the study involves determining the arsenic species present in the 3000 urine samples of study participants from Bangladesh. In addition, our collaborators have collected information such as the age, sex, and health outcomes of the study participants.
Ovarian cancer is ranked as the 5th most common cancer for women. Patients with advanced-stage ovarian cancer often experience recurrence as a result of resistance to first-line platinum-based chemotherapy. Resistance to platinum-based chemotherapy is only determined after tumor recurrence. Being able to distinguish platinum-resistant ovarian cancer before relapse would allow a personalized approach to patient treatment.

We used protein expression data from a cohort of 96 ovarian cancer patients included in The Cancer Genome Atlas (TCGA) databases. Differentially expressed proteins were first selected using the students’ t-test with p-values <0.05. The dataset was then divided into 5 subsets to perform external 5-fold cross validation. Feature selection with logistic regression was performed on each training set. Using this strategy, we identified 102 proteins that have predictive power with respect to platinum resistance.

We next compared different machine learning algorithms. The hyper-parameters of each model were determined by internal 5-fold cross validation and the optimal hyper-parameters were used. Accuracy was measured with the testing sets. Artificial neural network (ANN) gives the highest accuracy of 90% in distinguishing between patients with platinum-resistance from platinum-sensitive ovarian cancer with external 5-fold cross validation. Our ANN model can provide another piece of information in early determination of the platinum resistance status in ovarian cancer.

Our next goal will be to test our model on a larger scale of patient cohort and to reduce the amount of different proteins needed for this prediction model in order for our model to be more suitable for clinical use.
Cancer cells acquire resistance to MK-1775 through upregulation of Myt1

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The Cdk1/cyclin B complex is crucial for mitosis. Cdk1/cyclin B phosphorylates ~200 substrates leading to processes including chromosome condensation and nuclear envelope breakdown. During interphase, Cdk1/cyclin B is inhibited by the cell cycle kinases Wee1 and Myt1 which add inhibitory phosphates to Cdk1, preventing premature mitosis. When the cell is ready to enter mitosis, the inhibitory phosphates are removed by Cdc25 phosphatase. MK-1775 is a small molecule Wee1 inhibitor currently in phase I and II clinical trials against different cancers, including breast cancer. Inhibiting Wee1 with MK-1775 results in entry into mitosis with DNA damage leading to eventual mitotic catastrophe. Our data has shown that some breast cancer cell lines are resistant to MK-1775. Myt1 is a strong candidate for promoting resistance due to its redundancy with Wee1 in Cdk1/cyclin B inhibition. Using a crystal violet assay to measure cell viability under treatments with increasing concentrations of MK-1775, our lab found a correlation between Myt1 expression and MK-1775 resistance in several cell lines. My project aims to establish a relationship between Myt1 upregulation and reduced Cdk1 activity in the presence of MK-1775 by an in vitro kinase assay. We can measure Cdk1 activity by quantifying phosphorylation levels of a recombinant substrate (GST-PP1C-S) by western blot. Cells resistant to MK-1775 can be selected for in vitro by culturing sensitive cells in the presence of MK-1775 over ~60 days. We found that resistant breast cells (high Myt1 expression) had lower Cdk1 activity compared to sensitive controls (low Myt1 expression) in the presence of MK-1775. Furthermore, Myt1 can be overexpressed using a Flp-In T-Rex tetracycline inducible gene expression system. Cells with tetracycline induced Myt1 overexpression showed suppression of Cdk1 activity in the presence of MK-1775. My data supports Myt1 upregulation promoting resistance to MK-1775 in breast cancer through inhibition of ectopic Cdk1 activity.
Jingjie Xiao

Oncology

Stakeholder Engagement and Integrated Palliative Care in Canada

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Introduction. Palliative Care Matters (PCM) is a pan-Canadian initiative to improve access to quality palliative care services for all Canadians. Understanding the leadership, resources and interests of palliative care stakeholders in Canada will help engage organizations and more effectively work together. The objective of this study is to survey key stakeholders' characteristics and their capabilities in influencing palliative care policies. Methods. We employed a well-known stakeholder analysis methodology (Kammie Schmeer 1999) consisting of 8 steps. We first identified organizations and individuals with decision-making authority that have a key role in improving palliative care. Stakeholders were identified through their contributions to national references (i.e. reports, legislative bills and judicial court cases). Next, we utilized key informant interviews and online surveys to survey key stakeholders in order to understand how the 2018 Framework on Palliative Care in Canada informs their work. Investigated characteristics included knowledge, interest, position, leadership, alliance, power and resources. Results. Over 800 individual organizations contributed to 115 national reports (41 policy, 11 legislative, 63 judicial) over the last two decades. Prioritization resulted in 75 organizations eligible for survey, of which 54 responded. Factor and clustering analysis differentiated stakeholders into 4 engagement levels: highly engaged (n=18), engaged/Unsure of best approach (n=17), engaged/low priority (n=10), and somewhat engaged (n=7). Discussion and Conclusion. Stakeholder organizations contributing to national palliative care policy conversations throughout this period were broadly representative of society. The matrix analysis demonstrated that organizations vary greatly in their stated interests, resources and willingness to lead initiatives. Engagement strategies require resources and coordination. A stakeholder analysis is a useful method to understand each other’s interests and position. Further analysis and refinements are necessary for a national engagement strategy. By working together, duplication is avoided, novel opportunities are postulated and policy objectives are more likely to be realized.
Xia Xu

Oncology

Brain fatty acids binding protein and polyunsaturated fatty acids: Investigating their effect on glioblastoma membrane phospholipid composition and membrane fluidity

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Glioblastoma (GBM) is the most common primary brain malignancy. Brain fatty acid binding protein (B-FABP or FABP7) is associated with increased GBM cell migration and a poor clinical prognosis. Long chain polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and arachidonic acid (AA) are abundant in brain cell membranes. DHA and AA are preferred ligands of FABP7, with DHA having the stronger affinity for FABP7.

There are 4 main subclasses of phospholipids in cell membrane lipid bilayers: phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI). Recently, we used gas chromatography (GC) to compare the phospholipid fatty acid composition of two GBM tumour cultures derived from a patient: A4-004Ad cells (normal 'adherence-promoting’ condition) and A4-004Ns (neurosphere condition). Of note, A4-004Ns culture expresses much higher levels of FABP7 than A4-004Ad. We observed preferential incorporation of DHA into PC, PE and PS of A4-004Ns cells upon DHA supplementation compared to A4-004Ad cells. AA is preferentially incorporated into PC of A4-004Ns cells upon AA supplementation. We also used a quantitative fluorescence microscopy technique (Laurdan) to investigate FABP7-mediated DHA effect on membrane fluidity. Our results indicate DHA increased membrane fluidity in FABP7-positive cell lines compared to FABP7-negative cell lines.

Our results indicate that FABP7 expression in GBM cells affects the incorporation of DHA and AA in membrane phospholipids and membrane fluidity. We propose that these effects may underlie FABP7-mediated increased in cell migration upon increased AA:DHA ratio. We will further investigate the effect on phospholipases in DHA- and AA-treated GBM cells.
Cytomegalovirus infection increases breast cancer metastasis with the virus directly stimulating inflammatory responses in breast cancer cells

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Background: Cytomegalovirus (CMV) infects 40-70% of women. Infections are normally latent and only cause problems in immune-compromised and elderly people. Detection rates of >90% have been reported for CMV in breast tumors. CMV is suggested to be oncomodulatory in breast cancer. We studied the effects of CMV in mouse models of breast cancer and on cultured human breast cancer cells. We hypothesized that CMV infection induces inflammation, which promotes breast tumor progression.

Methods: Mice were latently infected with mouse CMV prior to breast tumor development. Tumor mass, phenotypes and metastasis were compared to uninfected mice. Human breast cancer cell lines were challenged with human CMV (HCMV) or UV-inactivated HCMV. Cells and medium were collected to detect inflammatory cytokine production. We also analyzed 86 human breast tumor samples for the HCMV genome.

Results: Latent CMV infection had no significant effect on breast tumor growth, but it enhanced lung metastasis. Tumors had increased metastatic phenotypes including more multiple-lobed tumors that contained more blood and less collagen. Infected mice also had increased plasma levels of IL-6 and IL-13 suggesting enhanced inflammation, and decreased tumor IL-1alpha, which could explain lower collagen levels.

mRNA expressions of cyclo-oxygenase-2 and IL-6 were increased when human breast cancer cells were challenged with HCMV for 36h. Breast cancer cells were not productively infected by HCMV, although the viral immediate early gene was transcribed from 3-12h.

HCMV genomes were detected by PCR in 86 human breast tumors and 7 breast tissue samples from women without cancer at 41% and 29%, respectively. This will be related to cancer stage and tumor genotype, once the remaining 70 samples are analyzed.

Significance: CMV infection promotes breast cancer metastasis and inflammatory cytokine production. HCMV does not infect breast cancer cells productively but it stimulates inflammatory cytokine production that could be oncomodulatory in breast cancer.
INTRODUCTION: DEAD Box1 (DDX1) protein is a member of a large family of DEAD box proteins comprising~40 members in humans. All DEAD box proteins have 12 conserved motifs, including the signature motif ‘D (aspartic acid)–E (glutamic acid) – A (alanine) – D (aspartic acid)’. DDX1 has multiple roles in mammalian cells including RNA transport, RNA processing and the repair of DNA double-strand breaks. Overexpression of DDX1 in some cancers, such as breast cancers, is associated with a poor prognosis. Ddx1KO mice are embryonic lethal, suggesting that DDX1 plays an essential role in early embryogenesis.

METHODS: We immunostained early stage embryos with different antibodies to DDX1 to determine the subcellular distribution of DDX1. We also used 5 Gy ionizing radiation to introduce double-strand breaks (DSB) in embryos and examined the role of DDX1 in these irradiated embryos. Gamma-H2AX was used as a surrogate marker of double-strand breaks.

RESULTS: We found that DDX1 is restricted to the cytoplasm of early stage embryos. This is in contrast to most mammalian cells tested to date where DDX1 is preferentially located in the nucleus. Furthermore, DDX1 localized to granules distributed throughout the cytoplasm of embryos, and RNA is required for retention of DDX1 in these granules. We also found that: (i) upon irradiation of 2-cell stage embryos, gamma-H2AX foci returned to baseline levels after 6 hours post-irradiation and, (ii) DDX1 remained in the cytoplasm of irradiated embryos, suggesting that DDX1 does not play a role in the repair of double-strand breaks in early stage embryos. We are currently in the process of examining recruitment of other DNA repair proteins to double-strand breaks in irradiated embryos. Our work to date indicates that phosphoATM (pATM), normally involved in the repair of DNA double-strand breaks by homologous recombination, is not be recruited to double-strand breaks in 2-cell stage embryos. We will pursue these analyses using antibodies to homologous recombination and non-homologous end joining to further investigate the repair of double-strand breaks in early stage embryos.
Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer that accounts for approximately 1–5% of all breast cancers but 10% of all breast cancer-specific mortality. Approximately, 20-30% of IBC patients are presented with distant metastasis at diagnosis which results in a lower overall survival rate in contrast to non-IBC. Despite improvements in identifying IBC as a different subtype of breast cancer, the intricate cellular mechanisms underlying the highly metastatic nature of this disease is still unknown. Thus, elucidating the biologic and molecular characteristics of IBC should aid in earlier diagnosis and improve patient overall outcome.

Given the role of the receptor tyrosine kinase (RIPK2) in many inflammatory diseases including cancer, I aim to understand the function of RIPK2 in IBC tumorigenesis. I hypothesize that RIPK2 can promote cell growth, invasion, metastasis, and cytokine increase. Utilizing IBC cell models and patient tumour samples, I have determined the presence of an increased level of active RIPK2 using western blot and immunohistochemistry, respectively. As a result of the activation of RIPK2 and its downstream signalling proteins, elevated production of pro-inflammatory cytokines and growth factors were found in IBC cell culture supernatant suggesting a contribution to molecular inflammation and metastasis of IBC.

To fully understand the importance of RIPK2 in my IBC cell model, I’m working on constructing stable Ripk2 knockout using Cas9/gRNA Ribonucleoproteins (RNP) genome engineering. Successful clones with Ripk2 deletion will be examined for (a) cell proliferation, (b) invasion, (c) metastasis and (d) cytokine level. All of these functional assays will elucidate the role of RIPK2 in the invasion-metastasis cascade that is known to correlate with cancer poor prognosis.
Tyrosine kinase inhibitors (TKIs) are effective in the treatment of non-small cell lung cancer (NSCLC) patients who harbor sensitizing mutations in the epidermal growth factor receptor (EGFR) gene. However, the T790M mutation in EGFR confers resistance to the first- and second-generation TKIs. EGFR mutations need to be tested for appropriately using targeted NSCLC treatment. Though tissue biopsy is used as a standard method for testing EGFR mutations, non-invasive liquid biopsy has been considered a low-risk alternative to tissue biopsy for EGFR detection. The status of EGFR mutations in liquid and tissue biopsies from the same NSCLC patient is expected to be compared to confirm whether liquid biopsy can be reliably used as an alternative approach for testing EGFR or not. A clinically validated qPCR was used for testing EGFR mutations in tissue biopsy samples of NSCLC patients. A next-generation sequencing-based assay (AVENIO ctDNA expanded kit, Roche) was used for testing EGFR mutations in plasma cell-free DNA (liquid biopsy) of NSCLC patients. Three pairs of tissue and plasma samples were tested in this study. The concordance between the EGFR exon 19 deletion testing results from plasma and tissue samples was 100% (3/3). However, EGFR T790M mutation status in plasma and tissue samples only showed 33.3% (1/3) concordance. Two pairs of samples only showed EGFR T790M mutation in tissue biopsy but not in liquid biopsy, indicating that tissue biopsy needs to be tested to confirm a negative EGFR T790M detection result from the liquid biopsy.
Janet Zhou

*Laboratory Medicine and Pathology*

**Studying the Influence of Selenium on Arsenic Hepatobiliary Transport with the HepaRG Cell Line.**

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Background: Over 200 million people worldwide are exposed to the proven human carcinogen arsenic at levels exceeding the World Health Organization guideline (10 µg/L). In animal models arsenic and selenium are mutually protective via the formation and biliary excretion of the seleno-bis (S-glutathionyl) arsinium ion \([\text{SeAs(GS)}_2]^-\). Despite ongoing human selenium-supplementation trials in arsenic endemic regions, the influence of selenium on human hepatic handling of arsenic is not adequately understood. In the liver, multidrug resistance protein 2 (MRP2/ABCC2) transports arsenic metabolites, including \([\text{SeAs(GS)}_2]^-\) into bile, and the related MRP4 (ABCC4) transports other arsenic metabolites into sinusoids. We hypothesized that selenium will increase the biliary excretion of arsenite (AsIII) from human HepaRG cells, an immortalized cell line used as a surrogate for primary human hepatocytes.

Objective: To study the influence of selenite (SeIV), selenide (SeII), methylselenocysteine (MSC) and selenomethionine (SM) on arsenic efflux from HepaRG cells.

Methods: The expression of genes involved in hepatic arsenic metabolism (As3MT) and export (ABCC2 and ABCC4) were assessed. Crude membrane preparations subjected to SDS-PAGE and immunoblots were used to evaluate the presence of MRP2 and MRP4 proteins. Fluorescence microscopy after treatment with 5(6)-carboxy,2',7'-dichlorofluorescein (CDF) diacetate was performed to visualize the canalicular networks and assess MRP2 function. Transport across sinusoidal and canalicular membranes were measured after treatment of HepaRG cells with 1 µM 73AsIII +/- SeIV, SeII, MSC or SM. Biliary excretion indices (BEIs) were calculated to quantify biliary excretion.

Results: ABCC2, ABCC4, and As3MT are expressed in the cell line, and MRP2 and MRP4 proteins were detected. CDF accumulation in canalicular networks suggested that MRP2 is functional. SeII increased biliary excretion of 73AsIII, with a BEI of 24%, but other forms of selenium did not.

Conclusion: This work will provide a better understanding of the influence of selenium on arsenic handling by human liver and provide valuable information for ongoing selenium-supplementation trials.
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