Impact of Loss of SLC43A3 on the Biodistribution and Metabolism of 6-mercaptopurine in Mice

Aaron L. Sayler, Hannah Dean, Chan S. Kim, James R. Hammond

Poster

Background: 6-mercaptopurine (6-MP) is a remission-inducing pharmacotherapy used for childhood acute lymphoblastic leukemia (ALL). We previously reported that the SLC43A3-encoded equilibrative nucleobase transporter (ENBT1) is largely responsible for the cellular accumulation of 6-MP. Therefore, differences in the tissue expression and activity of ENBT1 may impact both the therapeutic activity and adverse effects of 6-MP.

Methods: To explore the impact of ENBT1 on 6-MP actions and adverse effects, we have acquired a novel slc43a3 knockout (global) mouse model. 6-MP was administered to both knockout (KO) and wild-type (WT) mice via oral gavage (75 mg/kg). Mice were euthanized 90 minutes post-dose and a range of tissues were harvested. High performance liquid chromatography was used to assess the tissue levels of 6-MP and its metabolites.

Results: KO mice bred successfully and showed no gross morphological differences, nor differences in whole body or wet organ weights compared to WT mice. 6-MP showed significant decreased bioaccumulation in KO mice compared to WT mice in both males and females and across all organs examined. 6-MP metabolites also showed a significant decrease in bioaccumulation in the KO mice, particularly in tissues that normally had the highest expression of slc43a3, such as the liver, lungs and heart.

Conclusions: The loss of slc43a3 impacts the metabolism and biodistribution of 6-MP and its metabolites in mice. Heart, lung and liver may be particularly sensitive to changes in slc43a3 expression and may underlie adverse effects of 6-MP clinically observed in these tissues. Results suggest that loss of ENBT1 reduces the gastrointestinal absorption of 6-MP. Identical experiments are currently being conducted with azathioprine, the pro-drug of 6-MP to delineate any permeation differences between the two mainstay therapies.

Leukemia, IBD, 6-mercaptopurine, ENT1, knockout

Nano-delivery of pyronaridine as an inhibitor of DNA repair for targeted sensitization of head and neck cancer cells to cisplatin

Ahmed Abdelfattah, Parnian Mehinrad, James Donnelly, Michael Weinfeld, Frederick G. West, Afsaneh Lavasanifar

Poster

Background: Head and neck cancer (HNC) ranks seventh globally, with 660,000 new cases and 325,000 deaths annually. Nonsurgical treatments for HNC mainly include radiation therapy and platinum-based DNA damaging therapies. However, the ability of cancer cells to repair DNA damage can undermine the effectiveness of these treatments. ERCC1-XPF is a key enzyme in removing DNA damage caused by platinum therapies and radiation. Excitingly, our research team has found that the anti-malaria drug pyronaridine (PYD) can effectively hinder the dimerization and activity of ERCC1/XPF. The purpose of this study was to investigate the synergistic activity of free versus liposomal formulations of PYD in combination with cisplatin in FaDu cells, in vitro.

Methods: Liposomes were prepared through ethanol injection method followed by external buffer exchange and active loading of PYD in liposomes with an interior acidic pH. Cytotoxicity of cisplatin, PYD, or liposomal PYD as well as PYD formulation with cisplatin combination against FaDu cells was investigated by MTT and colony forming assay. The synergistic effect between the combinations was assessed using Combenefit software.

Results: The IC50 of cisplatin was significantly reduced when combined with 0.5 and 1 µM of PYD in FaDu cells. Furthermore, Combenefit analysis showed a synergistic effect between cisplatin (5-10) µM and PYD (1.2-1.4) µM. This synergy was further enhanced after using liposomal PYD, leading to wider effective concentration ranges and an augmentation in the magnitude of the observed synergy.

Conclusion: The results showed a synergistic cytotoxicity between PYD and its liposomal formulation with cisplatin against HNC.

Pyronaridine, Cisplatin, Head and neck cancer

Mathematical Modelling Non-Small Cell Lung Cancer Tumor Data

Alexandra Shyntar and Thomas Hillen

Poster

I will show that simple assumptions about tumor growth and response to treatment lead to a mathematical model that can explain tumor volume of non-small cell lung cancer (NSCLC). In our study, we examined data obtained from 23 patients diagnosed with NSCLC that received concurrent chemoradiotherapy, where the data set is composed of patients who respond and do not respond to treatment. We found that our model fits well to both responding and non-responding patients and there is significant variability in the resulting model parameters for patients. From our modelling results, we find a model-based biomarker that may indicate the patient's response to treatment, which may be used to improve the treatment regimen.

mathematical modelling, lung cancer, differential equations

Activin A in Cancer Cachexia: A Novel Role of Polymorphonuclear Myeloid-Derived Suppressor Cells

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Poster and presentation

Cachexia, a multifactorial syndrome associated with cardiac and skeletal muscle wasting, contributes to the death of about one-third of cancer patients. Several cytokines and cellular effectors were proposed to drive cachexia development; though, the exact mechanisms remain largely undetermined. Our lab projected polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) as a key player in mediating muscle wasting via their expansion to cardiac and skeletal muscles in lung cancer murine models, where depleting these cells protected against cachexia. Exploring mechanisms utilized by PMN-MDSCs through employing a PMN-MDSC-specific Cre-recombinase system revealed the important role of Activin A contrasting other mediators such as IL-6, TNF-α, and Arginase-1. We found PMN-MDSCs to be a major source of Activin A, which was increased in the serum of cachectic mice. Blocking Activin A signaling pathway and inhibiting its production by PMN-MDSCs prevented cardiac and skeletal muscle loss. Our findings indicate a critical immune-hormonal axis that opens the door toward developing novel therapeutic interventions for cachectic patients.

Cancer cachexia, Activin A, PMN-MDSCs

Accuracy of predictive equations against a gold standard measurement of resting energy expenditure in colorectal cancer survivors

Ana Paula Pagano, Iasmin Matias de Sousa, Sarah A. Purcell, Michael B. Sawyer, João Felipe Mota, Carla M. Prado

Poster and presentation

Background: Adequate energy intake is essential to optimize body weight and composition in cancer survivors. Accurate prediction of energy needs is a cornerstone for personalized nutrition care. We investigated the accuracy of resting energy expenditure (REE) predictive equations in colorectal cancer survivors (CRCS).

Methods: REE was measured in CRCS using a whole-room indirect calorimeter (WRIC) and compared with 22 predictive equations. Differences between predicted versus measured REE were tested using paired samples t-tests. Accuracy was described as estimations within 10% of WRIC-REE. Limits of agreement (LOA) were defined as mean error ± 1.96 SD and described individual level agreement. The relationship between body composition (assessed with dual-energy x-ray absorptiometry and computerized tomography) and REE was determined via Pearson correlation.

Results: Twenty CRCS were included (50% each sex; age: 61±14y; BMI: 28.8±6.4 kg/m2) 55% colon cancer and 75% had stage III cancer. Median time elapsed from end of treatment to study visit was 13.2 months (interquartile range: 21.2 months). Differences between measured (1697±331 kcal/day; range: 1182-2386 kcal/day) versus predicted REE were found for 86.4% of equations (n=19; all underestimations). Most equations accurately predicted REE in at least 50% of CRCS, with Harris-Benedict and Müller equations being the most accurate (65% of CRCS). Smallest LOA were found for Johnstone (-22.3 to 0.6%), and Harris-Benedict (-21.1 to 8.0%). Measured REE had a positive and very strong correlation with weight, fat-free mass, and skeletal muscle, and a significant positive and moderate correlation with BMI, fat mass, and visceral adipose tissue.

Conclusion: Most equations did not accurately predict REE at both group and individual levels. Measured REE was associated with weight, BMI, and body composition parameters.

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Predictive equations; whole-room indirect calorimeter; resting energy expenditure; energy metabolism; cancer.

Particle diffusion through heterochromatin domains is influenced by size and physicochemical properties

Anastasia Roemer, Michael Hendzel

Poster

The material state of chromatin is a subject of interest and controversy. We have recently shown that chromatin has solid (gel)-like properties consistent with many studies showing viscoelastic solid behavior of chromatin in cells. If chromatin is a viscoelastic gel pores will exist between crosslinked chromatin strands that limit the accessibility of molecules in a size-dependent manner. Single molecule tracking of nucleosomes has provided evidence for liquidity at the nanoscale while maintaining a gel state at the compartment scale. Heterochromatin proteins have been shown to form condensates that incorporate and concentrate chromatin in vitro. This has led to models where the heterochromatin itself is in a liquid state.

To begin testing this distinction, we probed heterochromatin for size-based accessibility, and found no clear correlation between the size of the proteins tested and their access to heterochromatin domains in mouse cells. Preliminary data with microinjected fluorescent secondary antibodies, which are about 10 nm in diameter, demonstrate that they can access heterochromatin domains. Interestingly, when we microinjected 20 nm carboxyl-modified beads, we found that they could not enter heterochromatin. This implies a size exclusion of molecules between 10-20 nm.

We did find evidence that the entry of proteins from the nucleoplasm into constitutive heterochromatin compartments is controlled. We find that EGFP is depleted from heterochromatin domains beyond volume exclusion effects. To further distinguish size and physicochemical exclusion effects from heterochromatin, we used small molecular weight cellular dyes that are not expected to be affected by size exclusion. We found that the dye Calcein AM is not depleted from heterochromatin, whereas the dyes Celltracker Orange CMRA, and 5-carboxyfluorescein are. Overall, our work is suggesting a more nuanced picture of particle diffusion through the nucleus, where inclusion or exclusion in nuclear domains is dependent on both the physicochemical properties and size of the particle.

Nuclear compartments, diffusion, heterochromatin

Role of TFAM in cGAS/STING Mediated Anti-Tumor Immunity of MSI Colorectal Cancer

Angie Chen, David Doell, Shayla Mosley, Kristi Baker

Poster

Colorectal cancer (CRC) is the third leading cause of cancer death in Canada. There are two subtypes of CRC characterized by genetic abnormalities called the microsatellite instable (MSI) and the chromosome instable (CIN) CRC. Patients with MSI CRC have greater survival compared to patients with CIN CRC due to an increased anti-tumor immune response that may be induced by the cytosolic DNA (cyDNA) sensors cGAS/STING. Alongside nuclear DNA, cyDNA also originates from mitochondrial DNA (mtDNA) which may strongly activate cGAS/STING due to its bacterial origin. Mitochondrial transcription factor A (TFAM) is a mitochondrial protein commonly truncated in MSI CRC that regulates mtDNA stability which may influence mtDNA release into the cytoplasm. We hypothesize that truncated-TFAM in MSI CRC will promote mtDNA activation of cGAS/STING through increased mtDNA release into the cytoplasm which then enhances the anti-tumor immune response. We first characterized the truncated-TFAM in MSI CRC. CRISPR-Cas9 modified MC38 mouse CRC cells made by Courtney Mowat were used to model the MSI and CIN subtypes. These cells were further modified with CRISPR-Cas9 to induce the truncated-TFAM into each subtype. MSI subtype models have successfully truncated TFAM as seen in western blots. qPCR quantification of mtDNA in isolated total DNA show reduced total mtDNA content in truncated-TFAM MSI CRC cells. Meanwhile, quantification of mtDNA in isolated cyDNA shows increased mtDNA content compared to wildtype (WT) TFAM controls, indicating greater mtDNA leakage from the mitochondria. Preliminary data shows an increased immune response in truncated-TFAM MSI CRC cells compared to WT TFAM controls. By understanding the TFAM truncation's involvement in immune activation in MSI CRC, we hope to uncover ways to boost immune responses in CIN CRC.

mitochondrial DNA, cytosolic DNA, colorectal cancer, immunology, mitochondria

Novel TFI-2-based PROTACs targeting the FOXM1 oncoprotein in breast cancer cells

Antonio Vega-Medina, Gloria Shi, Joanne Hadfield, Bahar Nematollahzadeh, Erika Loredo-Calderón, Gordon Chan, Adam Karpf, Carlos Velázquez-Martínez

Poster

The FOXM1 protein belongs to the Forkhead box protein superfamily of evolutionarily conserved transcription factors and it is considered an intrinsically disordered protein (IDP) due to the absence of ordered domains in the vast majority of its backbone, being the DNA-binding domain (DBD) the only region with ordered structure. FOXM1 plays an essential role in the regulation of several cellular processes such as cell proliferation, cell progression, cell differentiation, and apoptosis. However, its overexpression is directly involved in multiple stages of cancer progression including: (a) unregulated cell proliferation, (b) migration, (c) metastasis, (d) angiogenesis, (e) drug resistance and it is associated with poor prognosis in cancer patients. Due to its evident relevance in cancer, different approaches have been used to target FOXM1 either indirectly (targeting upstream mediators or inducing proteasomal degradation) or directly (targeting the DBD). Proteolysis targeting chimeras (PROTACs) are a new class of potential anticancer agents that induce degradation of a protein of interest (POI) by recruiting a E3 ubiquitin ligase. Then, the POI is polyubiquitinated and degraded by the 26S proteasome. In the present study, we tested novel FOXM1 TFI-2-based PROTACs (compounds 20, 22, 25), the 17d PROTAC (previously published by our group) and FOXM1 inhibitors (TFI-2 and F7) in different breast cancer cell lines using crystal violet viability assays, colony formation assays and western blots. Our results demonstrate that novel PROTACs lead to cell death, inhibition of colony formation, and decreased levels of FOXM1 to a greater extent than the 17d PROTAC or FOXM1 inhibitors, depending on the cell line. Further experiments are required.

FOXM1, PROTACs, Breast cancer

Binding of T cell immunoglobulin and mucin domain-containing protein-3 (TIM-3) with its ligand

Ashira Manzoor, Khaled Barakat

Poster

Purpose: TIM-3 is an immune checkpoint receptor that is expressed by various immune cells such as T cell, dendritic cells, natural killer (NK) cells, mast cells. TIM-3 has four ligands Galectin-9, Phosphatidylserine (PtdSer), CEACAM1 (Carcinoembryonic antigen related cell adhesion molecule), High mobility group protein B1 (HMGB1). Among them Galectin-9 has been identified as the first ligand of TIM-3. Interaction of TIM-3 and Gal-9 can suppress immune response and promote tumor growth. This study aimed to see the binding of TIM-3 and its ligand through a binding assay.

Methodology: In order to study the interaction of TIM-3 with its ligand we performed binding assay. For that we coated Gal-9, HMGB1, CECAM1 onto ELISA plates overnight. Biotin tagged rhTim3-6His-Avi-tag was added to the plates and detected with Streptavidin-HRP (horseradish peroxidase). TMB substrate was added to the plates. The absorbance was measured at 450- 570nm.

Results: A dose-dependent manner was observed in the binding of TIM-3 with its ligand. Among all the ligands Gal-9 showed the strongest binding.

Conclusion: Our study demonstrates that HMGB1, CECAM1 and Gal-9 are ligands of TIM-3.

TIM-3, Galectin-9, Immune checkpoint inhibitors, HMGB1

Genetic mutations at the Toll-like Receptor 4 informs on susceptibility to cisplatin-induced hearing loss in childhood cancer patients

Asna Latif, Erika Scott, Abhinav Thakral, Jong Lee, Geoff Liu, Bruce Carleton, Colin Ross, Amit Bhavsar

Poster

Cisplatin is a versatile chemotherapeutic that has been an indispensable tool in the treatment of a variety of cancers. However, it is limited by its toxicity. Over 50% of childhood cancer patients develop permanent hearing loss following treatment, leading to significant cognitive and socioeconomic impacts as well as necessitating a reduction of cisplatin doses during treatment. Interestingly, there is significant variation in susceptibility to cisplatin induced ototoxicity (CIO) from person to person, with some being very susceptible and others less so; this suggests a genetic component to CIO susceptibility. While several genetic targets have been proposed to be involved in toxicity, we recently found that cisplatin's interactions with an immune receptor, Toll-like receptor 4 (TLR4), mediate inflammatory responses by direct interactions. This suggests that genetic variation at the TLR4 locus may change susceptibility to CIO in patients. Thus, our study sought to investigate how mechanistic interactions of TLR4 with cisplatin could inform on genetic susceptibility to CIO.

To achieve this, candidate gene studies at the TLR4 locus were conducted in cisplatin-treated patient cohorts which revealed an association between single nucleotide polymorphisms in the TLR4 promoter and increased protection against CIO. Follow-up functional studies in-vitro confirmed that these mutations were associated with reduced promoter activity in the presence of cisplatin, suggesting decreased TLR4 expression. This indicates that the presence of these polymorphisms at the TLR4 promoter can be used as a predictive measure for CIO in clinic. By implementing genetic screening, we can personalize treatment regimens for patients and improve long-term health outcomes in cancer therapy.

Ototoxicity, pharmacogenomics, childhood cancer, immunology

Predicting energy requirements throughout breast cancer trajectory: what is the best equation?

Bruna Ramos da Silva, Ana Paula Pagano, Amy A. Kirkham, Maria Cristina Gonzalez, Mark J. Haykowsky, Anil A. Joy, Karen King, John R. Mackey, Ian Paterson, Edith Pituskin, Richard Thompson, Carla M. Prado

Poster

Introduction: Accurately estimating energy requirements is crucial for optimizing energy intake, particularly in the context of varying energy needs, like cancer. We sought to evaluate the agreement between resting energy expenditure (REE) predicted by equations versus measured REE in patients with breast cancer.

Methods: Data from four studies of patients with stages I-IV breast cancer were combined. Estimated REE from 30 predictive equations were compared with REE assessed by indirect calorimetry using a metabolic cart (MC) or a whole-room indirect calorimeter (WRIC). Agreement between methods was evaluated with Bland-Altman and Lin's concordance coefficient correlation (CCC).

Results: Ninety participants were included. Mean MC-REE and WRIC-REE were 1389 ± 199 kcal and 1506 ± 247 kcal, respectively. Limits of agreement were wide for all equations, including the most common ones; none had a bias within ±10% of measured REE, and all had a low agreement per CCC analyses (< 0.90). Korth equation had the best overall agreement compared to WRIC (range: 14.2 to -21.1%).

Conclusion: None of the evaluated equations accurately predicted REE at the individual level. Korth showed the best performance at the individual and group levels compared to the state-of-the-art WRIC. However, this equation still had significant inaccuracies for some patients. No breast-cancer-specific equations have been proposed to date. Equations specifically developed for these patients are needed to optimize energy requirements for those requiring personalized nutrition advice.

breast cancer, energy expenditure, energy metabolism, predictive equations, indirect calorimetry, whole-room indirect calorimeter

64Cu production via the 68Zn(p,nα)64Cu nuclear reaction: An untapped, cost-effective, high energy production route for local supply

Bryce J.B. Nelson, Melinda Wuest, John Wilson, Samantha Leier, Jonathan Doupe, Jan D. Andersson, Frank Wuest

Poster

Introduction: 64Cu (t1/2 = 12.7 h) is a high-resolution diagnostic PET imaging radionuclide ideally suited for theranostic applications with beta-emitting 67Cu for targeted radionuclide therapy. Our objectives were to assess radiochemical quality, radiotracer stability, and evaluate in vivo PET imaging of 64Cu produced via the 68Zn(p,nα)64Cu reaction. DOTA, NOTA, TETA, and PSMA I&T were radiolabeled with 64Cu, and these compounds were tested for in vitro stability. [64Cu]Cu-PSMA I&T was used for in vivo PET imaging studies to assess the quality of 64Cu synthesized via this production route and its suitability for application as a theranostic imaging partner alongside 67Cu therapy.

Results: A maximum purified activity of 4.9 GBq [64Cu]CuCl2 was obtained in 5 mL of pH 2-3 solution. HPGe gamma spectroscopy of the purified 64Cu product found <0.3% co-produced 67Cu at EOB with no other radionuclidic impurities. NOTA, DOTA, TETA, and PSMA I&T were radiolabeled with 64Cu, resulting in maximum respective molar activities of 164 +/- 6 GBq/umol, 155 +/- 31 GBq/umol, 266 +/- 34 GBq/umol, and 117 +/- 2 GBq/umol. PET imaging in LNCaP tumor-bearing mice resulted in tumor uptake with an SUVmean of 1.65 +/- 0.1 using [64Cu]Cu-PSMA I&T, while [68Ga]Ga-PSMA I&T yielded an SUVmean of 0.76 +/- 0.14.

Conclusions: 64Cu was purified in a small volume amenable for radiolabeling, with yields suitable for preclinical and clinical application. With a robust 64Cu purification process, and in vivo 64Cu PET imaging demonstrating excellent imaging performance, utilizing the 68Zn(p,nα)64Cu reaction is an attractive 64Cu production route for facilities with access to a higher energy proton cyclotron, compared to using expensive 64Ni target material and the 64Ni(p,n)64Cu reaction.

Advances in knowledge and implications for patient care: Our 64Cu production technique provides an alternative production route with the potential to improve 64Cu availability for preclinical and clinical theranostic studies alongside 67Cu therapy.

Cyclotron, Theranostics, Targetry, PET Imaging, Copper-64

The associations between docosahexaenoic acid (DHA) and carotenoids, exercise patterns, and quality of life in women undergoing neoadjuvant chemotherapy in the DHA WIN RCT

Claire Douglas, Susan Goruk, Kerry S Courneya, Catherine J Field

Poster

Cancer is a leading cause of death worldwide, with breast cancer serving as the most diagnosed type in 2020. Although neoadjuvant therapy is administered to patients to improve surgical resection outcomes and reduce micrometastases, not all individuals achieve a pathological complete response (pCR). Therefore, it is crucial to understand factors that may improve the efficacy and reduce the side effects of neoadjuvant chemotherapy.

Docosahexaenoic acid (DHA) is an omega-3 polyunsaturated fatty acid that has demonstrated antitumorigenic effects in preclinical models. It has also been shown to improve drug-induced side effects when administered during chemotherapy. This may in turn contribute to better patient outcomes, including exercise habits, specific dietary habits, and quality of life (QoL).

The current study is based on the completed phase II randomized controlled trial (RCT) evaluating DHA supplementation (4.4 g/day) in women with breast cancer undergoing neoadjuvant chemotherapy (DHA WIN). The objective is to investigate changes in exercise patterns, specific dietary habits, and QoL among participants.

Exercise has been associated with improved patient outcomes and QoL, as well as a reduction in chemotherapy-induced side effects. The average aerobic exercise of participants in the DHA WIN trial significantly decreased from timepoint one (129.6 minutes) to timepoint seven (72.7 minutes) ($p=0.03$). Significance was lost when stratified by treatment group. Groups did not significantly differ in their mean aerobic exercise per week at any of the seven timepoints. There was no significant association between the treatment group and meeting the World Health Organization's weekly aerobic exercise recommendations.

Specific dietary habits will be assessed by measuring plasma carotenoid levels, which are antioxidants that serve as biomarkers for fruit and vegetable intake. Various associations between exercise patterns, QoL, plasma carotenoid levels, pCR and treatment completion will also be investigated.

Breast cancer, docosahexaenoic acid, exercise

Single Cell RNA Sequencing Characterization of an in vitro Model for Studies of Hepatitis B Virus

Connie Le, Reshma Sirajee, Rineke Steenbergen, Michael A. Joyce, William R. Addison and D. Lorne Tyrrell

Poster

Purpose: Two billion individuals have been infected with hepatitis B virus (HBV) globally, resulting in 400 million chronically infected carriers. Unfortunately, chronic carriers can develop complications in spite of available treatments, culminating in approximately 1 million deaths annually. HBV chronic infection represents the leading cause of hepatocellular carcinoma worldwide. Although infectious in vivo, there is a lack of HBV infectable in vitro models. Conventional in vitro culture systems use hepatoma cells with sodium taurocholate co-transporting polypeptide (NTCP) overexpression, grown in medium supplemented with fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO).

Materials and Methods: We overexpressed NTCP in a liver cell line (Huh7.5 cells) and subsequently supplemented with human serum (HS). We characterized Huh7.5-NTCP cells differentiated in HS-supplemented culture using single-cell RNA sequencing (scRNA-seq). Single-cell RNA sequencing is a state-of-the-art technique that generates big data depicting RNA expression profiles of individual cells. Analysis of generated data was conducted using R programming language and the Seurat analysis package.

Results: In human serum cultures, HBV infection was enhanced by as much as 20-fold in comparison with conventional cultures supplemented with FBS with DMSO. Human serum culture increased levels of hepatocyte differentiation markers in Huh7.5-NTCP cells to similar levels found in primary human hepatocytes. Furthermore, N-glycosylation of NTCP induced by culture in human serum may contribute to enhanced viral entry. scRNA-seq analysis of 3119 Huh7.5-NTCP cells at the single cell resolution revealed heterogeneity within the cell culture system. Most HS-cultured Huh7.5-NTCP cells had similar gene expression profiles to those of primary hepatocytes isolated from human liver.

Conclusions: Importantly, a subgroup of cells expressed cholangiocyte-like phenotype. These results indicate that our HS-differentiated Huh7.5-NTCP hepatoma cell model is a useful alternative to primary human hepatocytes for studying HBV.

Hepatitis B virus, scRNAseq

The dynamic association of PARPs 1 and 2 with DNA double-strand breaks

Dan Fan, Zhigang Jin, Jean-Yves Masson, Guy Poirier, and Michael Hendzel

Poster

Poly(ADP-ribosyl)ation is a post-translational modification associated with a range of cellular processes, including DNA damage response (DDR). PARP family members, including PARP-1, PARP-2, and PARP-3, play significant roles in DDR. Among them, PARP-1 and PARP-2 can utilize nicotinamide adenine dinucleotide (NAD+) as substrates to synthesize poly(ADP-ribose) (PAR) chains which stimulate remodelling of the chromatin into a more accessible state for repair. The dynamic association of PARP1 and PARP2 to DNA damage sites has principally employed laser-induced DNA damage. The limitation of this approach is that it induces a broad spectrum of DNA damage types, including oxidized bases, single-strand breaks, and double-strand breaks. To better understand the association of PARPs specifically at DNA double-strand breaks, we have used an inducible restriction enzyme-mediated break-induced within an integrated lac operon array. Using this approach, We find that PARP2, but not PARP1, robustly recruits to the site of the DSB. Furthermore, we find that PARP2 localizes directly on the array using high-resolution fluorescence microscopy. We find that PARP2 can be recruited to DSBs. PARP inhibitor can induce PARP2 trapping, and PARG inhibitor can cause PARP2 slow exchange at DSBs. PARP2 has both PAR- and DNA-binding abilities, which are essential for the recruitment of PARP2 at DSBs. The N-terminal region (NTR) is responsible for PAR-binding, exhibiting rapid exchange. The DNA-binding ability is essential for PARP2 trapping. We will also present our results on the spatial relationship of PARPs to factors that participate in the signaling, processing, and homologous recombination repair machinery using this model DSB system.

PARPs, poly(ADP-ribosyl)ation, DNA damage response, double-strand break, dynamic

The role of the CXCR2 signaling on PMN-MDSCs in cancer cachexia.

Derek Parker, Kasia Dzierlega, Masoud Akbari, Sue Tsai, Xavier Clemente-Casares

Poster

Cancer-associated cachexia is a disease characterized by the loss of cardiac and skeletal muscle caused by a malignant growth; approximately 80% of late stage cancer patients will develop this disease. Work from our lab has shown that polymorphonuclear-myeloid derived suppressor cells (PMN-MDSCs) are immunosuppressive cells which are expanded under tumor burden that are necessary for cachectic wasting.

Comparison of the cytokine profiles of pro-cachectic versus non-cachectic tumor cells revealed that pro-cachectic tumor cells produce higher levels of several ligands of CXCR2, including CXCL1, 2, 3, and 5/6. For context, CXCR2 is a chemokine receptor expressed on myeloid cells which plays roles in neutrophil recruitment and function. We hypothesized that the CXCR2 signaling axis is essential for the cachectic process through its effects on PMN-MDSCs.

C57BL/6 mice were subcutaneously injected with 3.5 million Lewis Lung Carcinoma (LLC) cells, a pro-cachectic cell line. 4 days after tumor injection, mice were treated with CXCR2 inhibitors or DMSO every 2 days. 14 days after LLC injection, mice were euthanized and tissues were processed for cachexia assessment and flow cytometry. Inhibitors used included SCH-527123, a non-specific CXCR1/2 inhibitor, and SB225002, a specific inhibitor. Next, we generated reciprocal bone marrow chimeras using CXCR2 deficient mice. Mice with CXCR2-deficiency in either their hematopoietic cells or non-hematopoietic cells were injected with 3.5 million LLC cells. 14 days after LLC implantation, mice were euthanized and tissues were processed for cachexia assessment and flow cytometry.

Results from the inhibitor experiments showed that inhibition of CXCR2 rescues LLC tumor-bearing mice from cachexia. Additionally, chimeric mice with CXCR2-deficiency in their hematopoietic cells demonstrated no cachectic wasting. However, chimeric mice with CXCR2 deficient non-hematopoietic cells did demonstrate cachectic wasting. From this, we concluded that the CXCL1/CXCR2 signaling axis in PMN-MDSCs is essential for the development of cachexia.

Cancer, cachexia, immunology, muscle wasting

Improving reovirus as a oncolytic therapy for breast cancer by addressing cellular variation between different breast cancers

Dirk Taal, Maia Finch, Heather Eaton, Maya Shmulevitz

Poster

Oncolytic reovirus can be used to specifically target and destroy cancer without harming healthy cells. Ongoing phase II/III clinical trials using wildtype oncolytic reovirus (T3DPL) in breast cancer have shown minimal toxicity, but an underwhelming response rate, and therefore reovirus as a therapy for breast cancer needs improvement. Hard-to-treat breast cancers like Triple Negative Breast Cancer (TNBC) require new therapeutic approaches, so we strive to better understand the susceptibility of TNBCs to reovirus oncolysis. Immunofluorescence microscopy assessed two TNBC cell lines, EMT6 and E0771, for their susceptibility to T3DPL infection. EMT6 cells exhibited strong reovirus protein-specific immunofluorescence staining, indicating they were susceptible to T3DPL infection. Conversely, E0771 cells were poorly infected by T3DPL. Attachment of reovirus particles to EMT6 and E0771 cells was assessed at 4℃ to inhibit receptor-mediated endocytosis of the reovirus particles. Western Blot analysis identified that viral attachment was consistent between EMT6 and E0771 cells, suggesting that a decrease in binding did not result in the observed lack of susceptibility to T3DPL. When EMT6 cells exposed to T3DPL were incubated at 37℃ for two hours following attachment, cell-bound reovirus particles efficiently entered the cell, indicated by the prototypic lysosomal-dependent proteolytic processing of reovirus outer capsid proteins. Conversely, T3DPL particles attached to E0771 cells at 4℃ became detached from cells when incubated at 37℃ for two hours, demonstrated by lack of cell-associated virus and accumulation of virus in the media. Together these studies suggest that E0771 cells present a good model of TNBCs that require modifications to reovirus to enhance susceptibility and oncolytic activity. Strategies to overcome restricted entry of reovirus in E0771 cells are under development, including the exploration of reovirus variants that can take advantage of cellular differences.

Oncolytics, breast cancer, TNBC, virotherapy

The Role of MAEA in DNA Replication and Replication Stress Response: Implications for Cancer Therapy

Elham Zeinali, Fatemeh Mashyakhi, Taylor Lovsund, Rabih Abu Faraj, Amira Fitieh, Mark Glover, Kristi Baker, Ismail Hassan Ismail

Poster

Cancer treatment modalities, including radiation therapy, exploit the vulnerability of cancer cells to DNA double-strand breaks (DSBs). However, the DNA repair machinery safeguards normal cells from DSB-induced lethality, highlighting the need to decipher the intricate regulation of DSB repair to enhance the effectiveness of radiation therapy in cancer patients. Chromosomal translocations, a hallmark of cancer, often arise from DSBs, further underscoring the importance of understanding DSB repair pathways.

In human cells, DSB repair takes place through two main pathways, homologous recombination (HR) and error-prone non-homologous end-joining (NHEJ) that are carefully regulated to avoid the formation of chromosomal aberrations. Of the two types of DSBs, Single-ended DSBs (seDSBs) can be generated during replication by the collision of progressing replication forks (RFs) with various DNA lesions. seDSBs are preferentially repaired by HR. Processing of seDSBs by the NHEJ is a cytotoxic mechanism. In S phase, seDSBs ends are initially sequestered by the NHEJ heterodimer Ku to initiate NHEJ and impair HR. In seeking to find genes that influenced Ku removal, we identified an E3 ubiquitin ligase subunit MAEA (Macrophage-Erythroblast Attacher). Our findings indicate that MAEA is an essential component of HR. Under replication stress (RS), MAEA deficiency caused RF progression and stability defects. However, the precise mechanism by which MAEA promotes fork stability remains unresolved. We, therefore, hypothesize that MAEA is essential to promote the error-free repair of seDSBs and maintain the stability of RF under RS. This study aims to elucidate the role of MAEA in DNA replication and the cellular response to RS. Furthermore, we will explore the therapeutic potential of targeting MAEA to maintain genome stability in cells through preclinical animal studies. Understanding the multifaceted functions of MAEA in the context of DNA repair and genome stability holds promise for the development of novel strategies to enhance cancer treatment outcomes.

MAEA, Double-Strand Breaks (DSBs), Homologous Recombination, Replication Stress, DNA Repair

FOXM1-TARGETING PROTACs POSSESSING A FOLATE RECEPTOR LIGAND.

Erika Loredo Calderon, Antonio Vega, Adam Karpf, Carlos Velazquez

Poster

The use of Proteolysis-targeting chimeras (PROTACS) to target proteins involved in cancer development is a promising approach, but non-specific tissue distribution of PROTACs is a potential issue that needs to be solved. The folate receptor α (FOLR1) is one of the most well-defined targets for drug delivery, because it is highly expressed in many cancer types, including High Grade Serous Ovarian Cancer (HGSC), while normal cells have very low or no FOLR1 expression. Alternatively, FOXM1 transcription factor is one of many proteins that are abnormally and consistently upregulated in most human cancers; it is involved in cancer initiation, cancer development, and drug resistance. Due to the above, the main goal of this project is to synthesize and evaluate novel FOXM1-targeting PROTACS containing a) FOXM1 inhibitor (F7), b) linker, c) E3-ligase ligand (pomalidomide) and d) folate receptor ligand, that will confer specificity toward HGSC ovarian cancer cells.

PROTAC, Folate Receptor, FOXM1, Ovarian Cancer

OBSERVING IRE1 EXPRESSION AND ITS POTENTIAL AS A THERAPEUTIC TARGET FOR PITUITARY NEUROENDOCRINE TUMORS

Evan Yin, Masahiro Nezu, Motoyasu Satou, Tae Tateno, Naoko Inoshita, Frank van Landeghem, Constance Chik, Toru Tateno

Poster

Pituitary neuroendocrine tumors (PNETs) occur in almost 17 % of the population and they represent about 10% of all intracranial tumors. Management of aggressive PNETs is challenging due to the lack of reliable biomarkers and limited treatment options. Inositol-requiring enzyme 1 (IRE1) plays a significant role in the Unfolded Protein Response that triggers upon endoplasmic reticulum (ER) stress. Previous studies confirmed that increased IRE1 expression by ER stress is linked to cancer development. Inhibition of IRE1 has shown to downregulate downstream UPR factors, resulting in suppression of cancer cell growth. Most of the PNETs produce peptide hormones in the ER, suggesting the presence of activated ER stress. We hypothesized that IRE1 would be an effective target for treating PNETs.

To assess the role of IRE1 in PNETs, we conducted in vitro experiments by using rat growth hormone (GH)-producing cells (GH4 cells) and mouse adrenocorticotropic hormone (ACTH)-producing cells (AtT20 cells). We also analyzed IRE1 expression in seventy human PNETs by immunohistochemistry with the Allred scoring system. In GH4 cells, STF083010, an IRE1 inhibitor, reduced GH protein expression with suppressed cell proliferation. STF083010 also suppressed ACTH production and cell proliferation in AtT20 cells. Gene suppression of IRE1 by siRNA reduced ACTH expression. Our immunohistochemical analysis showed that IRE1a is expressed in all of the human PNET tissues. There were no significant differences in tumor size and invasiveness between tumors with high IRE1a expression (score 6-8) and those with modest levels (score 2-5). This was likely due to selection bias, as nearly all the samples were macro PNETs. It would be necessary to conduct further analyses using micro

PNET tissue.

Our data suggests that IRE1 could be a novel therapeutic target. Further analysis, including the effects of IRE1 inhibitor(s) on human PNET cells, would be necessary to support this.

Pituitary tumors, IRE1, ER stress, hormone production, cell proliferation

Identifying new aryl hydrocarbon receptor modulators using computational techniques

Farag E.S. Mosa, Mohammed A. Alqahtani, Mahmoud A. El-Ghiaty, Khaled Barakat, and Ayman O.S. El-Kadi

Poster and presentation

Purpose: The aryl hydrocarbon receptor (AhR) represents a ligand-dependent transcription factor pivotal for environmental sensing, integrating diverse biological cues to regulate intricate cellular responses. It exerts control over the expression of numerous genes, including those responsible for metabolizing enzymes such as cytochrome P450. AhR plays a crucial role in tumor cell initiation, promotion, progression, invasion, and metastasis. This study adopts a comprehensive approach that combines virtual screening with molecular dynamics simulations. The aim is to identify AhR modulators and gain insights into their interactions with the AhR binding domain.

Methods: The foundation of our research involved obtaining the crystal structure of the AhR-PAS-B domain and assembling a curated drug library. We subsequently devised a virtual screening protocol that incorporated both High-Throughput Virtual Screening (HTVS) and Standard Precision (SP) docking procedures. Post-docking, all compounds derived from the XP docking output underwent rigorous post-processing and re-scoring based on their binding energies within the receptor-ligand interface using the Prime/MM-GBSA module from Schrödinger package. Selected docked poses were then subjected to extensive classical molecular dynamics (MD) simulations. Following MD simulations, we conducted binding free energy calculations using the MM-PBSA method. Clustering analysis techniques were employed to elucidate the dominant conformations within each complex.

Results: Our virtual screening protocol proved effective in identifying approximately 100 compounds with docking scores and Prime/MM-GBSA module binding energies falling below -7.5 kcal/mol and -35 kcal/mol, respectively. Results from MD simulations and subsequent clustering analysis provided valuable insights into the stability of both the protein and the ligand, pinpointing the dominant conformation within selected complexes. Notably, selected complexes exhibited robust interactions within the PAS B binding pocket.

Conclusions: This study successfully identified multiple compounds with the capacity to bind to the AhR PAS B domain. Moreover, our research findings hold considerable therapeutic potential for addressing disorders associated with AhR.

AhR, PAS B, virtual screening, MD simulations

CDK-mediated PNKP phosphorylation regulates Okazaki fragment maturation and protects replication forks from EXO1-mediated degradation after replication stress

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Poster and presentation

Uncontrolled degradation and collapse of stalled replication forks (RFs) are primary sources of genomic instability, yet the molecular mechanisms for protecting forks from degradation/collapse remain to be fully elaborated. Here, we show that polynucleotide kinase-phosphatase (PNKP) localizes at stalled forks and protects stalled forks from excessive degradation. Loss of PNKP results in EXO1-dependent nucleolytic degradation of nascent DNA at stalled RFs. This mechanism is different from the BRCA2-dependent fork protection pathway, which protects stalled forks from excessive MRE11-dependent nucleolytic degradation. Loss of PNKP results in defects in RF progression and Okazaki fragment maturation (OFM). Mechanistically, we demonstrate that cyclin-dependent kinases (CDKs) phosphorylate PNKP during the S and G2 phases of the cell cycle in response to replication stress. We also identify several residues within the linker and the phosphatase domains as crucial contributors to PNKP phosphorylation. We establish that threonine to alanine mutations affect the function of PNKP in OFM and fork protection. Together, our data indicate that PNKP plays an unexpected role in the stability of stalled RFs.

Genome instability, DNA repair, PNKP, Replication stress, and Okazaki fragments

Deep learning-based autocontouring algorithm for non-invasive intrafractional tumour-tracked radiotherapy (nifteRT) on Linac-MR

Gawon Han, Keith Wachowicz, Nawaid Usmani, Don Yee, Jordan Wong, Arun Elangovan, Gino Fallone, and Jihyun Yun

Poster

Purpose: To develop a neural network-based tumour autocontouring algorithm with implementation of patient-specific hyperparameter optimization (HPO), and to validate its contouring accuracy using in-vivo MR images of liver, prostate, and lung cancer patients.

Methods: 2D intrafractional MR images were acquired at 4 frames/s using 3 T MRI from 11 liver, 24 prostate, and 12 lung cancer patients. A deep neural network based on a U-Net architecture was applied for autocontouring, and was further improved by implementing HPO using Covariance Matrix Adaptation Evolution Strategy (CMA-ES). Six hyperparameters were optimized for each patient, for which intrafractional MR images and experts' corresponding manual tumour contours were input into the optimization algorithm to find the optimal set of hyperparameters. The U-Net, modified according to the optimized hyperparameters, was subsequently verified by autocontouring the tumour in the 70 testing images per patient. To evaluate the algorithm's autocontours, Dice's coefficient (DC), centroid difference (CD), and Hausdorff distance (HD) were computed between the manual and autocontours. The performance of the algorithm was benchmarked against two standardized autosegmentation methods.

Results: The algorithm was able to perform HPO for each patient. The mean(standard deviation) DC, CD, and HD of the 47 patients were 0.92(0.04), 1.35(1.03) mm and 3.63(2.17) mm, respectively. This mean DC is comparable to the intra- and inter-observer variations in manual contouring previously found as 0.88(0.04) and 0.87(0.04), respectively. Compared to the two benchmarking autosegmentation methods, the developed algorithm achieved the best overall performance in terms of contouring accuracy and speed.

Conclusion: To perform nifteRT on Linac-MR, an autocontouring scheme has been developed by implementing HPO to a deep learning-based autocontouring algorithm, and its contouring performance was evaluated using in-vivo MR images of liver, prostate, lung cancer patients. The developed algorithm performs patient-specific HPO enabling accurate tumour delineation comparable to that of experts.

autocontouring, tumour tracking, linac-MR, hyperparameter optimization, CMA-ES

Inhibition of ZNF281 Effectively Reduces Tumor Growth and Metastatic Spread in Advanced Prostate Cancer

Guocheng Huang, Seyed Amirhossein Tabatabaei Dakhili, Maria Areli Lorenzana Carrillo, Runtai Chen, Subhash Das, Saymon Tejay, Alois Haromy, Evangelos Michelakis, John Ussher, Gopinath Sutendra, and Adam Kinnaird

Poster and presentation

Objective: Prostate cancer (PCa) is the most common internal malignancy in men and despite advances in treatment options, advanced PCa remains incurable. The Krüppel-type zinc-finger transcription factor ZNF281 has been shown to be involved in regulating embryonic stem cell differentiation, the epithelial-mesenchymal transition (EMT) process, and cancer cell stemness. The potential functions of ZNF281 on PCa progression remain unclear and may represent a new therapeutic target. The purpose of this study is to investigate the mechanism of ZNF281 on PCa progression and evaluate the potential therapeutic options for targeting ZNF281 in advanced prostate cancer.

Methods: We studied the ZNF281 protein expression statuses in PCa and normal tissue and cells. The nuclear ZNF281 expression levels of primary prostate tumors, metastatic pelvic lymph nodes, and normal pericarcinomatous prostate tissues from 15 patients were investigated by immunofluorescence staining. We studied PCa cell lines' proliferation, migration, and invasion assay by manipulating the ZNF281 expression level. Detailed molecular mechanisms of ZNF281 were investigated by Western Blot and RT-PCR. Statistical analysis included the t-test for categorical explanatory variables and continuous outcome variables.

Results: ZNF281 expression in metastatic PCa tissues is significantly higher compared to the primary tumor and normal tissue ($p<0.001$). Additionally, ZNF281 expression level in the metastatic tumor is positively related to that in the primary prostate tumor ($p<0.05$, $R2=0.33$). Furthermore, inhibition of ZNF281 either by siRNA or treated with a putative ZNF281 inhibitor reduced cancer cell proliferation, migration, and invasion abilities. Detailed molecular mechanisms analysis showed that ZNF281 regulates the expression of Androgen receptor (AR) and the EMT-related proteins including SNAIL ($p<0.05$).

Conclusion: ZNF281 is highly expressed in PCa metastasis. ZNF281 promotes PCa by AR-dependent signal pathway and AR-independent signal pathways. Inhibition of ZNF281 represents a potential therapeutic target for advanced PCa in future clinical applications.

Prostate cancer; New theraputic target; ZNF281; Androgen Receptor

Pro-inflammatory cytokines promote autotaxin production in mammary fibroblasts and conversion to a cancer-associated phenotype

Humayara Khan, Xiaoyun Tang and David N. Brindley

Poster

Breast cancer is the most prevalent cause of mortality by cancer-related deaths in women. Autotaxin (ATX) is a secreted enzyme that converts lysophosphatidylcholine to lysophosphatidic acid (LPA), which promotes cancer progression and metastasis by signaling through G-protein coupled receptors (LPAR 1-6). LPA signaling increases the secretion of inflammatory cytokines, which increase further ATX secretion in a feedforward cycle. This cycle is a rationale therapeutic target for decreasing cancer metastasis. Our group previously demonstrated that ATX is not derived from the cancer cells, but from stromal cells such as fibroblasts and endothelial cells. The cross-talk in the tumor microenvironment among cancer and stromal cells, plays a crucial role in tumor progression and metastatic spread. Cancer-associated fibroblasts (CAFs) are a major constituent of the tumor stroma that promotes tumor progression by secreting factors that reshape the extracellular matrix and mediate immunosuppression. However, the mechanisms by which cancer cells convert quiescent fibroblasts to CAFs are not widely understood. We observed that the quiescent mouse mammary fibroblasts expressed negligible amounts of ATX. Mimicking the inflammatory conditions of the tumor micro-environment, we stimulated the fibroblasts with the pro-inflammatory cytokines, TNF-alpha and IL-6. Both cytokines markedly induced ATX production, where IL-6 stimulation demonstrated a much higher and a stable induction. Simultaneously, TNF-alpha and IL-6 stimulation transiently induced the expression of the CAF marker, alpha-smooth muscle actin, in fibroblasts, indicating an acquisition of CAF phenotype. CAFs are emerging as attractive therapeutic targets because of the presence of unique biomarkers and their diverse effects on surrounding cells. Our study provides a basis for the role of inflammation in the reprogramming of fibroblasts to CAFs in the micro-environment of breast tumors. This phenomenon can provide future directions to design strategies that target the ATX-LPA-inflammatory cycle as a novel approach for the treatment of breast and other cancers.

Breast cancer, Autotaxin, metastasis, inflammation, cancer-associated fibroblasts

Docosahexaenoic acid supplementation during breast cancer neoadjuvant chemotherapy enhance the immune response towards a potential protective effect

Jaqueline Munhoz, Marnie Newell, Susan Goruk, Dhruvesh Patel, Sunita Ghosh, Anil Abraham Joy, Catherine J. Field and the DHA-WIN team.

Poster

Docosahexaenoic acid's (DHA) pleiotropic anti-cancer effects were demonstrated in pre-clinical models of breast cancer. However, the effects of DHA supplementation in breast cancer patients remain unknown. We aimed to assess the systemic immune function of DHA supplementation (4.4g/day) during 18 weeks of treatment in women with breast cancer in the neoadjuvant setting (WIN). Whole blood from patients was collected at the beginning of each chemotherapy cycle and analyzed at baseline, 12 weeks, and at the end of treatment. Compliance to supplementation was evaluated by fatty acid analysis using gas chromatography. Immunoassays were performed to measure plasma cytokines, immune cell phenotypes and immune function by ex vivo stimulation of peripheral blood mononuclear cells with phytohemagglutinin (PHA). Statistical analyses were performed using generalized estimating equations followed by pos-hoc analysis in SPSS. In the supplemented group, the composition of DHA in plasma phospholipids and total lipids in red blood cells (RBCs) increased by ~2-fold from baseline (both $P<0.0001$) and remained constant in the placebo group at the end of treatment. The frequency of T helper (Th) cells (CD3+CD4+) reduced in both groups over time ($P<0.007$). However, the frequency of T cytotoxic (CD3+CD8+) cells increased only in the DHA group ($P=0.031$), while the frequency of T regulatory cells (CD3+CD4+CD127-CD25+) only increased in the placebo group ($P<0.01$) compared to baseline. The frequency of Th1 cells (CD3+CD4+CD183+CD196-) and the frequency of Th2 cells (CD3+CD4+CD183-CD196-) remained unchanged in both groups. IL-2 plasma levels were increased only in the DHA group over time ($P=0.068$). Although ex vivo IL-2 secretion decreased in both groups, the reduction was 2-fold greater in the placebo group. These preliminary results suggest that DHA supplementation may increase the effector immune response while helping maintain the regulation of the immune function. Further analysis will help to understand and correlate these immunoregulatory effects with clinical outcomes.

Immune function, breast cancer, docosahexaenoic acid, cytokines, immune cell phenotype.

Identifying TLR4 signaling proteins associated with cisplatin-induced ototoxicity

Jason M. D. Lane, Shu Y. Luo, Olivier Julien, Amit P. Bhavsar

Poster

Cisplatin is a highly effective chemotherapeutic drug for treating solid cancers. Cisplatin is used to treat a wide range of cancers including lung, bladder, cervical, ovarian, and testicular cancers. While being used in ~20% of all cancer patients, cisplatin treatment has several adverse effects that limit its use and require dose reduction, or discontinuation of this life-saving chemotherapeutic. Notable cisplatin-induced toxicities include permanent hearing loss (ototoxicity) that occurs in ~35% of adults and ~50% of children treated with cisplatin. Cisplatin-induced ototoxicity (CIO) is caused by the damage and death of hair cells in the inner ear. These hair cells are important transducers of mechanical stimuli to electrical signals, but they do not replicate.

We identified that Toll-like receptor 4 (TLR4) was a critical mediator of cisplatin toxicity. TLR4 is best known for being an immune receptor that detects bacterial infection. Our data indicates that cisplatin binds to TLR4 in a distinct manner from the bacterial agonist lipopolysaccharide (LPS). Upon agonist binding, TLR4 activates multiple signaling pathways through the recruitment of adapter proteins, including the canonical AP-1, NF-□B, and IRF-3 effector pathways. Nevertheless, a hallmark of signal transduction is the transfer of phosphate moieties between signaling proteins within a given pathway.

Given the different mechanisms of binding between agonists, we hypothesize that the downstream signaling pathways will also show differences in activity with distinct phosphorylation patterns. We have shown broad differences in signaling caused by LPS and cisplatin on TLR4. We aim to identify specific targets of cisplatin signaling using mass spectrometry and phosphorylation site analysis. Using targets found through these methods, we will use small molecule inhibitors and genetic manipulation to examine each target and their effects on mitigating CIO.

cisplatin, ototoxicity, therapeutics, signalling, TLR4

Determinants of KMT5B dysfunction in neurodevelopment and glioma.

Justin W. Knechtel, Joanne D. Hadfield, and D. Alan Underhill

Poster

Mutations in the lysine methyltransferase KMT5B, which is responsible for dimethylation of lysine-20 on histone H4 (H4K20me2), have been documented in neurodevelopmental disorders (NDDs) and pediatric diffuse intrinsic pontine glioma (DIPG). Although these mutations are distributed over the length of the KMT5B protein, the only annotated region is the catalytic SET domain. We therefore used KMT5B structure-function and phylogenetic analyses to guide the identification of potentially important sequence features. This delineated a minimal segment that accounted for the normal distribution of KMT5B to pericentromeric heterochromatin and, unexpectedly, revealed the presence of a cryptic nucleolar localization determinant. Because key proteins are known to shuttle to nucleoli as part of a quality control process during cell stress, we evaluated how heat-shock affected localization of KMT5B. We observed a striking redistribution of KMT5B from pericentromeric heterochromatin to nucleoli, suggesting the underlying localization factors are unmasked under stress conditions. Further evidence linking KMT5B to environmental stress was apparent with the presence of a novel redox switch that may modulate cofactor binding and enzymatic activity. The functional relevance of this switch was underscored by its conservation in KMT5B orthologs from premetazoa, including C. limacisporum and S. rosetta. Last, we identified sequence features at the carboxy terminus of KMT5B that provided further functional clues. These included a short sequence motif found in proteins that interact with the Origin Recognition Complex and an acidic domain interdigitated with aromatic amino acids that is characteristic of chaperone proteins involved in histone octamer loading. Both are consistent with the known roles of KMT5B in DNA repair and replication, including replication stress. Overall, this work provides a critical framework to understand genotype-phenotype relationships associated with KMT5B alterations in NDDs and glioma and suggest that KMT5B distribution/activity during cellular stress likely contribute to its pathogenic roles in absence of genetic alterations.

Epigenetics, Fluorescence Microscopy, Cellular Stress

The role of cancer stem-like cells in AML relapse is dependent on the types and doses of therapeutics

Justine Lai, Claire Yang, Cecilia Shang, Joseph Brandwein, Raymond Lai, Peng Wang

Poster and presentation

Despite the recent therapeutic advances for acute myeloid leukemia (AML), most patients die from disease relapse which occurs shortly after the initial remission. We recently reported that cancer stem-like (CSL) cells, detectable based on their SORE6 reporter activity, contribute to AML relapse after cytarabine (Ara-C) treatment in an in-vitro relapse model. Here, we asked if CSL cells contribute to AML relapse over a range of doses and types of treatments. In addition to Ara-C, we included azacitidine (AZA) alone and AZA plus venetoclax (AZA+Ven), two regimens used for patients who cannot tolerate Ara-C. Using two AML cell lines, treatment with Ara-C, AZA or AZA+Ven led to 'zero viability' on day 2, with no viable cells detected by trypan blue. In-vitro relapse (IR) was first detected on day 8 with Ara-C and AZA, whereas no IR was observed with AZA+Ven. Cells treated with lower drug doses resulted in IR with AZA+Ven and earlier IR with Ara-C/AZA. Using molecular barcoding, we found that IR after Ara-C was attributed primarily to the expansion of CSL cells, but the importance of CSL was observed only when 'zero viability' was achieved; the expansion of bulk cells was found to contribute to IR even with a slight reduction of the Ara-C dosage. With AZA and AZA+Ven, the contributions of CSL cells, which were variable even when 'zero viability' was achieved, were negligible at lower doses. Using metabolomic analysis, we found upregulation of ornithine decarboxylase (ODC) as a marker of CSL cell expansion. By western blot, we found the expression of this marker correlates with the expansion of CSL cells. Our findings suggest that the mechanism underlying relapse may be dependent on dose and type of treatment. This concept, if proven, may carry significant therapeutic implications for patients.

Acute myeloid leukemia, relapse, cancer stem-like cells

Chronic CMV infections produce cytokine changes in organs that could contribute to increased breast cancer metastasis.

Kaitlyn Visser, Xiaoyun Tang, David N. Brindley, Denise G. Hemmings

Poster

Our group showed that ~63% of Canadian breast cancer patients are latently infected with cytomegalovirus (HCMV). This is associated with a higher risk of stage IV metastasis. In two mouse models of breast cancer, latently mCMV-infected BALB/c or MMTV-PyVT mice had increased number and size of lung metastases without a change in breast tumor size. After initial active infection, HCMV establishes a lifelong latent infection whereby limited genes are expressed with no active replication. While acute infections change the cytokine profiles in organs, the long-term impact of chronic infections and the resulting effect on metastasis is less understood. We aimed to elucidate how latent CMV infection could contribute to increased metastasis.

CMV is associated with fibrosis, which increases lung metastasis. Thus, we hypothesized that pro-fibrotic cytokines would be increased in lungs and liver from latently-infected mice. We found 10/32 cytokines were increased in the lungs and 10/32 were decreased in the livers of latently-infected female BALB/c mice that did not have cancer. Surprisingly, pro-fibrotic cytokines IL-13 and IL-17 and the anti-fibrotic IL-7 were all increased in the lungs.

Latently-infected MMTV-PyVT mice with cancer also had increased pro-fibrotic cytokines IL-6 and IL-13 in plasma. Overt fibrosis was not observed since it takes longer to manifest; however, these pre-fibrotic changes could contribute to increased metastasis.

To test the metastatic impact of cytokine changes in the lungs, we injected 4T1 cells through the tail vein in latently-infected and uninfected BALB/c mice. However, no differences in metastases were found. This indicates that mCMV-induced changes in the primary tumor play an important role in increased metastasis.

Marked changes in liver and lung cytokines were observed in latently-infected mice, which demonstrates that infection has profound long-term effects. These could predispose to fibrosis, modify immune functions long-term and precondition these sites to entry and growth of metastases

Cytomegalovirus, Metastasis, Breast Cancer, Infection

IL-5 promotes pro-cachectic PMN-MDSC functions in cancer-associated cachexia

Kasia Dzierlega, Mainak Chakraborty, Derek Parker, Megan Lee, Masoud Akbari, Daniel Winer and Xavier Clemente-Casares

Poster and presentation

Background: Cancer cachexia is a metabolic disorder characterized by the progressive and irreversible loss of cardiac and skeletal muscle in response to malignant growth. This muscle-wasting disorder is evident in approximately 30-50% of cancer patients and accounts for one-third of all cancer deaths. Management of cachexia is a major challenge, and current treatments typically have shown limited effectiveness. Despite the growing list of implicated cachectic factors, little is known about the potential role of immune cells in this process.

Methods: We have characterized two tumor models, pro-cachectic Lewis Lung Carcinoma (LLC), and non-cachectic Colon Adenocarcinoma (MC38) implanted in C57BL/6 mice.

Results: Comparing these models revealed a significant expansion of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) in the hearts and muscles of cachectic mice. Depletion of PMN-MDSCs with aLy6G antibodies prevented cardiac and skeletal muscle loss. A comparison of a broad range of cytokines produced by pro-cachectic tumors using Luminex multiplexing showed that LLCs produced high quantities of PMN-MDSC-related factors, including IL-5, a Colony Stimulating Factor 2 Receptor Subunit Beta (CSF2RB) ligand. CSF2RB deficiency prevented the development of LLC-induced cachexia, without affecting PMN-MDSC expansion. Neutralization of IL-5 with anti-IL-5 antibodies completely abrogated cardiac and skeletal muscle loss. Furthermore, mice treated with an IL-5Rα inhibitor, preventing IL-5 signaling, were protected against both cardiac and skeletal muscle atrophy.

Conclusions: Collectively, our data suggest an important pathway involved in the development of cancer cachexia, specifically the expansion and IL-5/CSF2RB-dependent programming of pro-cachectic PMN-MDSCs.

Cancer, Cachexia, ROS, IL-5, Immunology

VEGF-C sensitizes lymphatic endothelial cells to DNA damage and ROS through activation of the VEGFR-3

Lazina Hossain, Karina Pereira Gomes, Xiaoyan Yang, Jacques Du Toit, Spencer B Gibson.

Poster

Many cancer survivors develop lymphedema due to damage to the lymphatic system caused by surgery, radiation therapy, and chemotherapy. This damage induces stresses such as oxidative stress in lymphatic vessel impairing the lymphatic system. In response to damage, vascular endothelial growth factor C (VEGF-C) levels increase to induce lymphangiogenesis leading to lymphatic cell proliferation to repair the lymphatic system. Unfortunately, high VEGF-C levels often fails to repair the damage and the reason is unknown. To determine the effect of oxidative stress, I treated Human Dermal Lymphatic Endothelial Cells (HDLEC) and Human Umbilical Vein Endothelial Cells (HUVEC) with different concentrations of H2O2. I found that 500uM H2O2 induced significant endothelial cell death, determined by trypan blue exclusion assay. Oxidative stress induces several different types of cell death. I found that H2O2 induced apoptotic cell death determined by AnnexinV/7-AAD assay but failed to induce autophagy determined by a lack of increase in LC3-II levels in HDLEC cells. Since VEGF-C is upregulated in cancer related lymphedema, I determined that VEGF-C increases proliferation normally but induced apoptotic cell death in the presence of H2O2. In addition, VEGF-C increased ROS levels in HDLEC and HUVEC cells following H2O2 treatment. Antioxidant pretreatment rescued HDLEC cells from VEGF-C induced cell death under oxidative stress. I also found that VEGF-C increased DNA damage detected as γH2AX by intracellular staining in the HDLEC cells under oxidative stress. Finally, I found that VEGFR-3 (VEGF receptor-3) inhibitor induced HDLEC cell death at high concentration, but blocked VEGF-C induced oxidative cell death at low concentration. These results indicate that VEGF-C sensitizes lymphatic cells to DNA damage and ROS leading to cell death through activation of the VEGFR-3. This could contribute to the reason why VEGF-C fails to repair the lymphatic system and may provide future treatment strategies to prevent lymphedema.

VEGF-C, VEGFR-3, Lymphatic endothelial cell, DNA Damage, Flow cytometry.

Investigating the Molecular Mechanisms of Breast Cancer Metastasis: A 3D Culture Model Perspective

Leila Pirayeshfard, John Maringa Githaka, Lucy Luo, Arashdeep Saini, Nicolas Touret, Olivier Julien and Ing Swie Goping

Poster and presentation

Cancer is the leading cause of death in Canada, with more women being diagnosed with breast cancer than any other type of cancer. Migration of these cancer cells to distant organs is the major cause of death from disease. Understanding cancer cell metastasis at a molecular level will undoubtedly provide tools for improved clinical outcome. Our lab identified the protein BAD as a prognostic marker for breast cancer patient survival. BAD is regulated by phosphorylation and using a genetic mouse model wherein 3 key phosphorylation sites are mutated to alanine (BAD3SA), we demonstrated that BAD3SA inhibits mammary epithelial cell migration, delays mammary gland development, and inhibits breast cancer metastasis. This study investigates the cellular and molecular mechanisms by which BAD modulates cell motility. Notably, anti-metastatic BAD3SA localizes to the mitochondria, whereas wild-type BAD is cytosolic. Targeting wild-type BAD to the mitochondria inhibits cell motility, indicating a significant role for mitochondria in this process. Furthermore, BAD3SA only inhibits migration of cells cultured in 3D but not in 2D. This suggests that BAD3SA regulates 3D-specific cell motility processes, possibly related to cell-extracellular matrix interactions. To gain molecular insight, we used a BioID biotinylation-labeling system (mini-Turbo biotin ligase) and identified BAD3SA-binding proteins in 3D culture by mass spectrometry. Pairwise comparison of experimental and control sample identified significant hits that were analyzed by unsupervised hierarchical clustering. Sixty BAD3SA candidate interactors were identified. Pathway analysis revealed significant roles in PlexinD1 signaling, Basigin interaction, and Integrin family cell surface interactions—consistent with a cell motility phenotype. Top candidates will be independently confirmed and functionally validated by pharmacological/genetic inhibition. This will identify molecular players that mediate the BAD effect on migration. Together, this work may uncover new cellular processes and druggable targets that inhibit breast cancer metastasis.

BAD, Breast cancer, Metastasis, Cell motility, Proteomics, 3D models

A New Mechanism A New Mechanism for N-terminal Dependent Protein Degradation

Lina Alhourani, Richard Fahlman

Poster

Protein degradation is essential for maintaining the intact proteome under both healthy and stressed conditions. Disruption of proper protein degradation could result in the accumulation of hazardous intracellular aggregated species and eventually diseases.

Proteins starting with Met are considered stable degrons, but some will still be degraded by the different branches of the N-end rule depending on the amino acids following the initiator Met. I found a new type of N-terminal-dependent degradation using Hela cells, and genetic tools to mutate the N-terminal part of expressed proteins, I noticed that proteins expressing MK- and MR- N-terminal motifs showed the least stability of translated reporters. To test if this type of degradation is a proteasomal-dependent type I treated the cell model with a proteasome inhibitor that was able to block the degradation. I tested two exogenous reporters expressing the same N-termini in comparison to a control. I was able to show that this type of degradation is not substrate-based dependent.

There is limited knowledge regarding such type of N-terminal-dependent degradation. Therefore, my project aims to investigate this type of N-terminal-dependent instability in mammalian cells, and determine their mechanisms of recognition. Together this study aims to reveal a novel mechanism that regulates the stability of proteins commencing with MK/MR. Determining the proteasomal involvement and identifying new enzymes that aid in the recognition process may uncover druggable targets for future therapeutic implications.

Protein degradation, N-End Rule pathway , MiniTurbo , Gel electrophoresis, Cell culturing

Examining the role of XBP1s expression in macro PNETs and assessing its usefulness a novel therapeutic target

Louisa Lu, Motoyasu Sato, Masahiro Satou, Frank van Landeghem, Naoko Inoshita, Constance Chik, Toru Tateno

Poster

Pituitary neuroendocrine tumors (PNETs) are mostly benign and progress slowly. Most of them are cured by surgery or controlled by medical therapy and/or radiation. We have limited management options for these aggressive PNETs with resistance to multi-modal therapies due to the lack of reliable biomarkers and limited treatment options.

XBP1s is a transcription factor for several genes, including oncogenes, activated by enhanced endoplasmic reticulum (ER) stress via IRE1a. A recent study demonstrated that XBP1s regulates secretory capacity of the pituitary gland. Inhibition of IRE1a-XBP1s pathway has been reported to suppress cancer cell growth. However, the role of XBP1s in PNETs remains unknown.

We assessed the role of XBP1s in PNETs, using rat mammosomatotroph tumor cells, GH4 cells, and mouse corticotroph cells, AtT20 cells as in vitro models of PNETs. Also, we analyzed XBP1s expression in 85 human PNET tissues by immunohistochemistry. XBP1s is expressed in GH4 cells and AtT20 cells. Pharmacological inhibition of the IRE1a-XBP1s by STF083010 reduced hormone production and cell proliferation in GH4 cells and AtT-20 cells. Our immunohistochemical analysis of XBP1s expression in human PNET tissues with the Allred scoring system showed that 66 samples have high XBP1s expression and 19 samples have moderate expression. More females ($p=0.003$) and more occurrences of the optic chiasm compression ($p=0.04$) were seen in the high XBP1s expression group. There was no significant difference in tumor size ($p=0.34$) and invasiveness assessed by the Knosp score system ($p=0.065$). This would be likely due to selection bias, as all the samples were larger than 1 cm in size.

Our data suggests that XBP1s could be a novel biomarker/therapeutic target. However, further immunohistochemical analysis using the normal pituitary gland and micro PNET tissues and primary cell culture studies are required to validate our findings.

XBP1s, pituitary tumours, novel therapeutics, optic chiasm compression

Lysosome disrupting agent Siramesine and venetoclax gives synergistic apoptotic response in primary CLL cells mediated by inhibition of autophagy

Madhumita Manivannan, Xiaoyan Yang, Nirav Patel and Spencer B. Gibson

Poster

Venetoclax, an antagonist of anti-apoptotic Bcl-2, is becoming a standard treatment for Chronic lymphocytic leukemia (CLL) patients. Unfortunately, many patients develop resistance against venetoclax. A strategy for overcoming drug resistance in CLL would be targeting different cellular mechanisms in combination with venetoclax treatments. Lysosomes maintain cellular homeostasis by regulating various events including the catabolic pathway through autophagy. The number of lysosomes in CLL cells are increased compared to normal B cells. Indeed, siramesine, a lysosome disrupting agent selectively induces cell death in CLL cells and gives synergistic apoptotic responses when combined with ibrutinib, a BTK inhibitor. It is unknown whether siramesine affects autophagy or gives synergistic apoptotic response when combined with venetoclax in CLL cells. In this study, we obtained CLL cells from patients at the Cross Cancer Institute and found that the combination of siramesine and venetoclax synergistically induced cell death in primary CLL cells compared to the control and individual drugs alone. This combination of drugs also increased lysosomal membrane permeabilization and reactive oxygen species (ROS). The combination also increased the cathepsin-D leakage by permeabilizing lysosomal membrane. In addition, siramesine treatment increased LC3-II levels which could be due to the autophagosomes failure to fuse with these degraded lysosomes. To indicate whether autolysosomes are reduced, we treated cells with chloroquine that blocks the fusion of lysosome with autophagosome and determined that LC3-II levels did not further increase with addition of siramesine. Furthermore, siramesine treatment failed to increase co-localization of LC3-II and LAMP1 in immunostaining of CLL cells. In conclusion, the increased cell death by combining siramesine and venetoclax could be due to increased LMP mediated by inhibition of autophagy in CLL cells. Future investigations will determine the extent of cell death in normal B cells and using FDA approved lysosome disrupting agents in combination with venetoclax.

Autophagy, Chronic Lymphocytic Leukemia, Combination Therapy, Lysosome, BCL-2 inhibitor

Overcoming constraints to reovirus oncolysis with σ1 variants

Maia Finch, Adria Clark, Dirk Taal, Paris Brown, Tim Footz, Dr. Heather Eaton, and Dr. Maya Shmulevitz

Poster

Although reovirus serotype 3 Dearing/PL lab strain (T3D-PL) is well tolerated by cancer patients in clinical trials, the effectiveness of T3D-PL virotherapy is low and requires improvement. We predict that T3D-PL oncolysis faces two main constraints involving the σ1 cell attachment protein: 1) Zinc-dependent metalloproteases secreted by breast cancer cells into the tumor microenvironment cleave σ1, significantly reducing T3D-PL infectivity. 2) Neutralizing anti-reovirus antibodies (NARAs) target the immunodominant σ1 protein, potentially limiting the oncolytic activity of reovirus boosts. To overcome σ1 cleavage by breast cancer metalloproteases and reovirus neutralization by σ1-directed NARAs, reovirus variants with metalloprotease-resistant and antigenically-distinct σ1 proteins representing three reovirus serotypes (T1, T2, and T3) were generated. S1 genes encoding σ1 were chosen from public sequence databases as representing the most diverse sequences among the three serotypes. These S1 genes, which naturally possess a mutation permitting metalloprotease resistance, were cloned into the plasmid-based reovirus reverse genetics system and used to produce 12 recombinant σ1 variants on an otherwise T3D-PL genetic background. Immunoblot analysis revealed that all 12 σ1 proteins were detected by their respective serotype 1, 2, or 3-specific polyclonal antibodies with minimal cross-reactivity. As a proxy for in vitro oncolytic activity, plaque size was measured on tumorigenic TUBO cells: 10/12 variants had reduced fitness relative to T3D-PL. Polyclonal antibody serums were generated in mice against the most oncolytic variant in each serotype. Linear and conformational σ1 epitopes resisted cross-reactivity to these serums. The most oncolytic variant in each serotype was tested in a standard murine breast cancer model. All three σ1 variants reduced tumor volume and prolonged survival relative to PBS-treated mice. Although this model revealed that the three σ1 variants possess in vivo oncolytic activity, it should be altered in future studies to better understand the impact of using antigenically-distinct reovirus therapies on oncolytic outcome.

breast cancer, oncolysis, neutralizing antibodies

A hybrid PCA acceleration method for rapid real-time MRI

Mark Wright, Gawon Han, Jihyun Yun, Eugene Yip, B. Gino Fallone, Keith Wachowicz

Poster and presentation

Purpose: To develop a temporally robust MRI reconstruction method suitable for low-latency real-time imaging for use in image-guided radiotherapy.

Methods: This work introduces a method by which sparsely-undersampled k-space is estimated in real-time through an analysis of the time-course and structural detail of the core k-space acquired over time. This core k-space refers to the central (low-frequency) data which is acquired in every frame. The outer (high-frequency) k-space data is undersampled in such a way that all of k-space is acquired in a pre-determined number of frames. Principal Component Analysis (PCA) is performed on the acquired data of the previous frames with the most relevant principal components (PCs) kept for reconstruction. These first PCs are representative of the core and outer k-space and are fitted to the acquired core data of the final frame to populate the missing data. A second reconstruction is performed to add stability to the final solution by performing PCA on the core data along the time axis. Again, the relevant PCs are fit to the acquired outer k-space data to better estimate the un-acquired data in the final frame.

Results and Discussion: Testing was performed retrospectively on 15 lung, 10 liver and 10 prostate data sets. The lung and five liver patients were analysed for image quality, while 10 lung and all liver and prostate data sets were analysed for the ability to contour, using an in-house built u-net based auto-contouring software, a structure within the image at a variety of acceleration rates. Results were found to be good with image quality metrics such as structural-similarity consistently above 0.9 and normalised-mean-square-error below 0.03. Contouring metric dice-coefficient was found to be greater than 0.89 for all sites and accelerations tested.

Conclusion: It was found that images reconstructed using this method were of sufficient quality to be contoured in real-time even at an acceleration factor of 8x.

MRI, real-time, target-tracking, image-guided, radiotherapy

Novel allosteric inhibitors targeting SHP2 in cancer

Maryam Jama, Marawan Ahmed, Anna Jutla, Carson Wiethan, Jitendra Kumar, Tae Chul Moon, Frederick West, Michael Overduin and Khaled H. Barakat

Poster

Purpose: The src homology region 2 (SH2) containing protein tyrosine phosphatase 2 (SHP2) is an oncoprotein and an emerging target for cancer treatment1. Its overactivation is associated with tumour initiation, progression, and metastasis. Current research is focused on developing allosteric inhibitors targeting SHP22. Potent inhibitors such as SHP099 paved the road for this research. However, despite extensive efforts, SHP2 allosteric inhibitors have yet to reach the clinic. Identifying highly bioactive and selective SHP2 inhibitors will be necessary for drug development and to better understand SHP2 functions in malignancies3. This study aimed to use computational modelling to identify novel SHP2 allosteric inhibitors and validate their activity through in vitro biochemical assays.

Method: Using in silico modelling, we screened over 6 million compounds against the allosteric site of SHP2. The compounds with the lowest free binding energy were selected for experimental testing using protein thermal shift assay (PTSA). Subsequently, the binding affinity of the compounds to SHP2 was confirmed using biolayer interferometry (BLI) assay. Next, the dose response of the three top compounds was investigated using DIFMUP as a substrate in a biochemical SHP2 inhibition assay. Lastly, the top compound's effect on the peripheral blood mononuclear cells (PBMC) was investigated using enzyme-linked immunosorbent assay (ELISA).

Results: In silico modelling identified 26 top ranked hits with < -28kcal/mol. The PTSA validated three compounds (C8, B9, C2), which exhibited a positive change in melting temperature. C8, B9, C2, and SHP099 were confirmed to bind to and inhibit SHP2's activity in the nanomolar range. Furthermore 100 nM of C8 significantly reduced various pro-inflammatory cytokine secretion.

Conclusion: Our study demonstrates an effective screening methodology for identifying active SHP2 allosteric inhibitors. The identified compounds demonstrated strong affinity and potent inhibition of SHP2 activity, enabling further investigation of SHP2 in cancer cell lines.

Phosphatase, Allosteric inhibitor, Binding assay,Protein thermal shift assay, in silico screening

Role of a testicular germ cell-specific fatty acid-binding protein in breast cancer

Mitchell Semeniuk, Rongzong Liu, Roseline Godbout

Poster

Fatty acid-binding protein (FABP) gene FABP12, the newest member of the FABP family, is normally expressed in testicular germ cells. We have recently found that FABP12 is amplified and overexpressed in human cancers, such as prostate cancer and breast cancer.

In breast cancer, FABP12 is preferentially enriched in triple-negative and luminal B tumours, subtypes with relatively worse clinical outcomes. Patient cohort analysis showed that high FABP12 levels are significantly associated with poor prognosis. Using triple-negative (HCC1806) and Luminal B (BT-474) cell lines, we found that siRNA depletion of FABP12 results in reduced colony formation in both cell lines. Cell proliferation was suppressed in BT-474 cells along with induction of p53 and inhibition of E2F1 upon FABP12 knockdown. We also showed that depletion of FABP12 results in attenuated cell migration but enhanced cell proliferation in HCC1806. In the ER-positive BT-474 cells, we demonstrated significant reduction of both PPAR element (PPRE)- and estrogen receptor element (ERE)-driven luciferase activity, accompanied by enhanced cell sensitivity to tamoxifen treatment upon FABP12 depletion. Importantly, depletion of FABP12 in BT-474 cells led to diminished fatty acid uptake and lipid accumulation in HCC1806, and reduced ATP production upon suppression of CPT1A, a rate-limit enzyme in fatty acid β-oxidation in mitochondria. Furthermore, we observed FABP12-mediated activation of NFkB, an oncogenic factor typically involved in tumour cell survival, inflammation and treatment resistance.

In conclusion, our initial in vitro studies of FABP12 in breast cancer cells indicate that: (1) FABP12 is amplified and ectopically expressed in breast cancer and associated with poor prognosis; (2) FABP12 may play roles in cell survival, migration and treatment resistance in triple-negative and/or luminal B breast cancers; and (3) the mechanisms underlying FABP12 function in breast cancer may involve aberrant lipid metabolism, activation of fatty acid-mediated signalling pathways and crosstalk with NFkB and ER-mediated oncogenic pathways.

FABP12, Breast Cancer, Fatty Acids, Cell Migration, Cell Proliferation

Exploring the effect of exercise on arm lymphedema: insights from MRI

Mona M. Al Onazi, Richard Thompson, Margaret L. McNeely

Poster

Background: Breast cancer-related lymphedema (BCRL) is a significant morbidity affecting one in five breast cancer survivors. It presents as swelling in the arm, breast and chest wall on the side of the breast cancer. BCRL can alter the tissue composition and lead to increased accumulation of adipose tissue and fibro-sclerotic tissue. Recent studies in BCRL have demonstrated that resistance exercise can improve symptoms without worsening lymphedema. No studies, however, explored the potential of combining resistance exercise with therapeutic strategies to help reduce arm lymphedema volume.

Purpose: To (i) discuss the impact of lymphedema on tissue composition and compare the data from clinical lymphedema measurements and MRI scans; (ii) discuss the impact of exercise on lymphedema using MRI.

Methods: A case study analysis was conducted to compare lymphedema measurements before and after the intervention. Lymphedema assessment involved the use of volumetric measurements and MRI.

Results and conclusion: There is a potential benefit of exercise on arm lymphedema and tissue composition. The underlying changes in tissue composition associated with lymphedema are not reflected in the clinical volumetric measurement.

Breast cancer, Lymphedema, Assessments, Exercise, MRI

Augmented CD8+ T Cell-Mediated Anti-Tumor Response via Leupaxin Deletion

Nafiseh Ghobakhloo and Hanne L. Ostergaard

Poster and presentation

Background: CD8+ T cells, particularly the effector-memory (Tem) subset that includes T effector cells, are vital for tumor response due to their potent cytotoxicity, tissue infiltration, and migration. Leupaxin, a cytoskeletal adaptor protein expressed primarily in leukocytes, including CD8+ T cells, plays role downstream of integrin and T cell receptor signaling. However, the precise function of leupaxin in T cells remains enigmatic. The primary objective of this study was to elucidate the contribution of leupaxin to the anti-tumor response mediated by CD8+ T cells.

Methods: Leupaxin-deficient mice (LKO) were generated from leupaxin-floxed mice (Lfl/fl) and injected with syngeneic tumor cells including EG7, EL4, and E0711. EG7 are EL4 T lymphoma cells expressing ovalbumin to increase immunogenicity. E0711, a breast adenocarcinoma, induces an immunosuppressive tumor microenvironment with limited tumor-infiltrating CD8+ T cells. Tumor cells were subcutaneously or orthotopically implanted in LKO and Lfl/fl mice (8, 12, and 16 weeks old), and tumor size was measured. CD8+ T cell frequency and phenotype in various compartments (tumors, livers, and spleens) were assessed via flow cytometry.

Results: In 12- and 16-week-old LKO mice, tumor growth was significantly suppressed across all models compared to Lfl/fl mice. Tumors in LKO mice exhibited increased infiltration of CD8+ T cells with effector/memory phenotypes. Notably, a distinct NK1.1-expressing subset within the Tem population was more abundant in LKO tumors compared to Lfl/f tumors. This NK1.1+ CD8+ T cell population is known for its potent anti-tumor properties, as demonstrated in both human and mouse studies.

Conclusion: Our finding suggests that leupaxin loss leads to increased unique population of Tem CD8+ T cells, that accumulates with age and enhances tumor elimination and offers a promising treatment target.

Leupaxin, NK1.1+CD8+ T cells, Anti-tumor response

203Pb labeled panitumumab for SPECT imaging of EGFR+ head and neck cancer

Nasim Sarrami, Bryce Nelson, Samantha Leier, John Wilson, Conrad Chan, Jalna Meens, Teesha Komal, Laurie Ailles, Raymond Reilly, Frank Wuest and Afsaneh Lavasanifar

Poster

Introduction: Radiotheranostics are traceable radiolabelled compounds targeting cancer biomarkers for application in cancer diagnosis and treatment. Epidermal growth factor receptor (EGFR) is one of these biomarkers which its recognition as an oncogene has led to the development of anticancer therapeutics directed against EGFR, including the human IgG2 monoclonal antibody panitumumab.

The objective of this research was to develop a new radiotheranostic based on 203Pb-panitumumab and assessment of its potential as an immuno-SPECT probe. Application of 203Pb (t1/2:51.9 hours) as the radiotracer is expected to provide an optimum timeframe for the imaging of solid tumors. Besides 203Pb has a complementary radionuclide (212Pb) which can be substituted with 203Pb and used for therapeutic purposes.

Methods: 2'-(4-(2-amino-2-oxoethyl)-10-(2-((4-isothiocyanate benzyl)amino)-2-oxoethyl)-1,4,7,10 tetraazacyclododecane-1,7-diyl) diacetic acid (PSC-NCS) reacted with panitumumab (pH=8, room temperature) then purified by size exclusion chromatography and its composition was confirmed by matrix-assisted laser desorption/ionization (MALDI). Radiolabeling with 203Pb was done at a pH of 4.5, room temperature for 5 minutes and the incorporation efficiency was measured using radio-TLC. 203Pb-panitumumab (~10MBq;140µl) was injected into NRG mice with subcutaneous head and neck squamous cell carcinoma PDX (n=5) via vein tail. SPECT/CT images were acquired 48 and 120 hours post-injection. For biodistribution studies, mice were euthanized 5 days after injection with 203Pb-panitumumab; tissues were collected, weighed, and γ-photons were counted via γ-counter. The uptake was calculated as injected dose percentage per gram of each tissue (ID%/g). Blocking experiments were performed by pretreating mice (n=5) with 1 mg of unlabeled panitumumab 1 hour before receiving 203Pb-panitumumab.

Results:~5 PSC chelators were attached per antibody and radiolabeling efficiency was 99.2+0.7%. The 203Pb-panitumumab homed and accumulated in the tumor (26% ID%/g) significantly higher than other organs (<6.2 ID%/g).

Conclusions: labeling with 203Pb was successful and reproducible;203Pb-panitumumab accumulated in EGFR+ tumor; a suitable immuno-SPECT probe for imaging EGFR+ tumor.

203Pb, SPECT/CT, heand and neck cancer, nuclear medicine imaging, panitumumab

Understanding the Cellular Response and Spatial Organization of DNA Double Stranded Break Repair Structures

Natnael Abate and Michael Hendzel

Poster

DNA double-stranded breaks (DSBs) represent a significant threat to the stability of the genome. Cells respond to DSBs by activating checkpoints in the cell cycle and recruiting DNA repair proteins to repair the damaged DNA. One crucial player in this process is Ataxia telangiectasia mutated (ATM) kinase, which phosphorylates histone H2AX at serine 139 to generate γH2AX. γH2AX then aids in recruiting additional signaling molecules, especially E3 ubiquitin ligases, that control the recruitment of two key proteins, 53BP1 and BRCA1. 53BP1 promotes Non-Homologous End Joining repair, whereas BRCA1 promotes Homologous Recombination for DNA repair. Our study, using analytical transmission electron microscopy, has revealed that the interior of these DSB sites is rich in proteins but lacks chromatin, suggesting that the DSB is located at the periphery of this compartment. Our research aimed to explore the relationship between the DNA undergoing repair and the sub-compartments within DSB repair structures. Previous research, as well as our findings, suggest that the organization of 53BP1 and BRCA1 in IR-induced breaks changes throughout the cell cycle, aligning with their respective roles in DNA repair. By utilizing the LacI-Fok1 system, we can pinpoint the location of the DSB and delve deeper into the organization of components involved in DSB repair. Through the LacI-Fok1 system, we observed three distinct sub-compartments within the repair structure: the damaged DNA, single-stranded DNA, and the nuclease CtIP, all localized within the interior of the compartment. BRCA1 and 53BP1 occupied specific regions within this compartment, with one overlapping the Lac array and the other positioned towards the periphery. Additionally, we found Rad51 and other proteins, were distributed in larger volumes than the array and occasionally were less concentrated within the array. These results shed light on the organization of key players responsible for executing and regulating DSB repair within the repair structure.

DNA Double Strand Breaks, non-homologous end joining (NHEJ), homologues recombination (HR), nuclear compartmentalization

Regulation of equilibrative nucleoside transporter subtype 2 (ENT2) by casein kinase 2 (CK2)

Nayiar Shahid and James Hammond

Poster and presentation

Background: Equilibrative nucleoside transporters (ENT1, ENT2) mediate the transmembrane flux of endogenous nucleosides and nucleoside-analogs used in anti-cancer (gemcitabine, cytarabine) and anti-viral (ribavirin, and zidovudine) therapies. Given the emerging evidence that ENT2 has distinct physiological roles from ENT1, an understanding of how ENT2 function is regulated in its native cellular environment is critical to controlling/exploiting its impact on cancer therapies using nucleoside analogues.

Objectives: In vitro studies and mass spectrophotometry have identified ENT2 phosphorylation by CK2 (casein kinase 2) at the consensus sites- Ser270, Ser282, and Thr285. To examine the effect of CK2 inhibitor, CX-4945 (silmitasertib, an anti-cancer drug) on ENT2 function and to assess the underlying mechanisms, we have developed and characterized a novel HEK293 mutant (using CRISPR/cas9) lacking ENT1 and expressing only ENT2 (HEK293-ENT1KO).

Methods: ENT2 function was assessed by measuring the rate of [3H]2-chloroadenosine (15 s time point) uptake (1–225 µM) ± CX-4945 (100 nM) using both short term (15) and longer-term treatment (24 & 48 hr) at 37°C in serum free media. Protein levels were assessed by immunoblotting using ENT2-specific antibodies.

Results: HEK293-ENT1KO cells had similar level of ENT2 uptake as wild-type HEK293 cells. Treatment of HEK293-ENT1KO cells with CX-4945 resulted in a significant increase in Vmax of ENT2-mediated uptake from 0.8 ± 0.1 to 2.1 ± 0.2 pmol/µl/s and Km shift from 23 ± 5.5 to 97 ± 18 µM (n=6, $p<0.0001$, 2-way ANOVA, Tukey's multiple comparisons post-test).

Conclusions: ENT2 activity is upregulated by CK2 in HEK293-ENT1KO cells suggesting an increased ENT2 trafficking to the plasma membrane, or decreased internalization from the membrane. The Km shift might be due to ENT2 conformational change leading to alteration in substrate binding pocket. Further exploitation of this new cell model may reveal mechanisms of ENT2 regulation and identify novel modulators of ENT2 expression and function.

CRISPR-Cas9, ENT2, regulation, casein kinase 2, silmitasertib

The Next Generation Trial: A validating paired-cohort trial assessing diagnostic accuracy of 18F-PSMA-1007 positron emission tomography versus magnetic resonance imaging in the primary staging of prostate cancer

Nikhile Mookerji, Guocheng Huang, Amaris Hui, Tyler Pfanner, Benjamin Adam, Peter Dromparis, Yuan Gao, Tarek Bismar, Stacey Broomfield, Patsy Branton, Lucas Dean, Patrick Albers, Wendy Tu, Christopher Fung, Alexander Tamm, and Adam Kinnaird

Poster and presentation

Introduction: Prostate specific membrane antigen (PSMA) is a type II transmembrane protein which demonstrates overexpression in most prostate cancers and correlates with tumor aggressiveness. PSMA positron emission tomography (PET) imaging has been shown to be superior to conventional imaging (CT/Bone scan) in the workup of metastatic prostate cancer. The objective of this study is to determine the accuracy of 18F-PSMA-1007 PET compared to the gold standard MRI in the primary locoregional staging of intermediate and high-risk prostate cancer.

Methods: The Next Generation Trial (NCT05141760) is a prospective phase II study assessing 18F-PSMA-1007 PET and mpMRI for locoregional staging of clinically significant prostate cancer in men undergoing radical prostatectomy. The study is a validating paired-cohort with final histopathology as the gold standard comparator thereby providing Oxford Level of Evidence 1b. Reviewers for each technique (MRI/PET) were blinded to preoperative and other imaging data. Standard sensitivity and specificity analysis was conducted followed by McNemar's test to compare groups.

Results: Between March and July 2023, 275 patients were assessed for eligibility. 150 patients were enrolled and had their scans completed. 14 were excluded after their scans due to opting for radiation (10) or having nodal metastasis (4). 66 patients have had their blinded results reported thus far. 29/66 (44%) patients had their PSMA PET be concordant with the overall pathological stage versus 15/66 (23%) using MRI (p=0.02). PSMA PET correctly identified bilateral disease in 44/66 (67%) versus 29/66 (44%) by MRI (p=0.03). Extra-capsular extension was correctly identified by PSMA PET in 46/66 (70%) vs 36/66 (54%) by MRI (p=0.04).

Conclusion: From this interim analysis, prostate cancers were more accurately staged by PSMA PET compared to MRI. As more blinded imaging and pathology results are compiled, this initial data will be further clarified and the quality of evidence increased.

PSMA PET, Prostate Cancer, Prostate MRI

Muscle and adipose wasting despite disease control: unaddressed side effects of chemotherapy for advanced pancreatic cancer

Pamela Klassen, Vickie Baracos, Sunita Ghosh, Michael B. Sawyer, Vera Mazurak

Poster

Muscle and adipose wasting during chemotherapy for advanced pancreatic cancer (aPC) are associated with poor outcomes. We aimed to quantify the contributions of chemotherapy regimen and tumour progression to muscle and adipose wasting and evaluate the prognostic value of each tissue loss. Of all patients treated for aPC from 2013-2019 in Alberta, Canada (n=504), computed-tomography (CT)-defined muscle and adipose tissue index changes (ΔSMI, ΔATI, cm2/m2) were measured for patients with CT images available both prior to and 12 ± 4 weeks after chemotherapy initiation (n=210). Contributions of regimen and tumour response to tissue change were assessed with multivariable linear regression. Survival impacts were assessed with multivariable Cox's proportional hazards models. Tissue changes varied widely (ΔSMI: -17.8 to +7.3 cm2/m2, ΔATI: -106.1 to +37.7 cm2/m2) over 116 (27) days. Tumour progression contributed to both muscle and adipose loss (-3.2 cm2/m2, p<.001, -12.4 cm2/m2, p=.001). FOLFIRINOX associated with greater muscle loss (-1.6 cm2/m2, p=.013) and GEM/NAB with greater adipose loss (-11.2 cm2/m2, p=.002). Greatest muscle and adipose losses were independently associated with reduced survival (muscle: HR 1.72, p=.007, adipose: HR 1.73, p=.012, tertile 1 versus tertile 3). Muscle and adipose losses are adverse effects of chemotherapy and may require regimen-specific management strategies.

pancreatic cancer; muscle wasting; cachexia; FOLFIRINOX

MICROULTRASOUND IN CANCER – ACTIVE SURVEILLANCE (MUSIC-AS)

Patrick Albers, Betty Wang, Stacey Broomfield, Anaïs Medina Martín, Wendy Tu, Christopher Fung, and Adam Kinnaird

Poster

Background: Accurate assessment of tumor grade is critical for prostate cancer (PCa) Active Surveillance (AS). Multiple new technologies, including targeted biopsies and advanced imaging techniques like multiparametic magnetic resonance imaging (MRI) and high-resolution micro-ultrasound (microUS) may improve tumor risk stratification.

Objective: To compare MRI and microUS for the detection of Gleason Grade Group ≥2 during AS.

Methods: Prospective, paired diagnostic trial of 210 men with Gleason Grage Group 1 PCa managed by AS undergoing confirmatory biopsy between 12/2022 and 09/2023 at an academic tertiary care centre. To date, 89 men have been consented for the study and 54 have undergone their confirmatory biopsies and have their pathology results available.

Outcome Measurements and Statistical Analysis: The primary outcome is the difference in detection rate of Grade Group ≥2 found using microUS + systematic biopsy and using MRI/US Fusion + systematic biopsy. Statistical analyses will be performed using Chi square test, Fisher's exact test, and McNemar test.

Results: Of the 54 men biopsied thus far, the average age of the participants was 62.1, with a median PSA of 7.1, and 25 (46%) with family history of prostate cancer in first degree relatives. 38 (70%) of the men had a PRI-MUS score ≥3, and 30 (56%) had a PI-RADS score ≥3. Gleason Grade Group ≥2 was identified in 24 (44%) men. There was no difference in the detection of Gleason Grade Group ≥2 between the imaging techniques, with all cancers detected by microUS + systematic biopsy and using MRI/US Fusion + systematic biopsy. One limitation of this trial is the single centre nature, though two international sites will ultimately be involved in our study.

Conclusions: The detection of upgrading to Gleason Grade Group ≥2 during AS appears similar when using microUS or MRI to inform prostate biopsy.

Prostate Cancer, Micro-ultrasound, Targeted Biopsy

Quantitative proteomics analysis reveals the prevention of chemotherapy induced catabolic changes by omega-3 fatty acids in a preclinical model of colorectal cancer

Peter Isesele, Richard Fahlman and Vera C. Mazurak

Poster and presentation

Cancer and chemotherapy each alter metabolism and cause muscle wasting, which is associated with poor prognosis. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to be beneficial to muscles during cancer therapy; however, the mechanisms are not well understood. This study aimed to investigate the effects of dietary EPA+DHA following standard chemotherapy treatment [(irinotecan +5-fluorouracil); FOLFIRI] on muscle using quantitative proteomics analysis.

Female Fischer 344 rats bearing the Ward colon tumor were fed a semi-purified diet resembling a westernized diet. Rats without tumors served as a healthy group (REF) and were handled similarly. After 2 weeks of tumor growth, FOLFIRI was administered, and rats were randomly assigned to remain on the control diet (CHEMO) or switched to a diet containing EPA+DHA (2.3 g/100 g of diet in the form of purified fish oil), initiated on the first day of FOLFIRI treatment (CHEMO + Fish oil). Rats were euthanized on Day 0 (TUM) prior to FOLFIRI administration and 8 days later. Protein was extracted from gastrocnemius muscle and was subjected to Proteomics Analysis using LC-MS/MS. The Differentially Expressed Proteins (DEP) using Fold-change cut-off ≥ 1.5 and P-value <0.05 were subjected to KEGG pathway analysis and Gene Ontology (GO) for functional enrichment.

In the CHEMO vs. REF, 329 DEP were identified, while Fish oil prevented many of these changes, with 44 DEP identified compared with CHEMO. Comparing the CHEMO with the TUM and REF group, the top significant ($p<0.05$) pathways include reactive oxygen species, proteosomes, PPAR signaling, and lipid transport. The top significant pathways in the CHEMO + fish oil vs. CHEMO were oxidative phosphorylation, 2-Oxocarboxylic acid metabolism, and citric acid cycle. GO enrichment reveals the induction of catalytic activities and cellular processes by CHEMO, while CHEMO + Fish oil reduced the number of proteins involved in these processes by 80% and instead seemed to promote energy utilization.

Proteomics analysis provides insights into the molecular mechanisms by which dietary EPA+DHA prevents chemotherapy-induced catabolic changes in the muscle and reveals the potential to mitigate the deleterious effects of chemotherapy on the muscle that confer poor prognosis

Cancer, Proteomics, Muscle Wasting, Chemotherapy

Drug repurposing screen on patient-derived preclinical models of dedifferentiated endometrial cancer identifies cardiac glycosides as a potential therapy

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Poster

Dedifferentiated endometrial cancer (DDEC) occurs when a low-grade endometrioid-type cancer abruptly transforms into undifferentiated cancer that lacks evidence of Mullerian epithelial differentiation. Genomic inactivation of core SWItch/Sucrose Non-Fermentable (SWI/SNF) complex proteins (i.e., ARID1A/1B co-inactivation, SMARCA4 inactivation) have been implicated in dedifferentiation in endometrial cancer. More than half of DDEC presents as advanced stage (III-IV) disease with a median disease-specific survival of 4.5 months and is resistant to conventional platinum/taxane-based chemotherapy. Its aggressive clinical course limits the opportunities for additional lines of therapy or clinical trials. We developed three patient tumor-derived cell line (3D spheroid) and xenografts (PDX) models (two ARID1A/1B co-inactivated and one SMARCA4 inactivated) that recapitulated the phenotypic and molecular profiles of DDEC. Given the aggressive nature of DDEC, its lack of response to standard-of-care chemotherapy and the limited opportunity to evaluate the efficacy of other anti-cancer drugs clinically, we propose to perform high throughput drug repurposing screen on these established models with the hypothesis that other clinically approved anti-cancer drugs will be more effective for treating DDEC. High-throughput in vitro screen on the SMARCA4-inactivated DDEC cell line using an 1800 compound FDA-approved drug library identified multiple cardiac glycosides, including digitoxin and digoxin. Further evaluation of identified drug candidates using in vitro CellTiter-Glo 3D cell viability confirmed the suppression activity of both digitoxin and digoxin with lower IC50 in DDEC compared to non-DDEC endometrial cancer cell lines. Additionally, the cardiac glycosides were tested using xenograft mouse models of DDEC-1. Initial results support the inhibitory activity of both digitoxin and digoxin in vivo in DDEC-1. Considering the safety profiles and the feasibility of assessing drugs from the drug repurposing screen it appears to be a rational approach to identify therapeutic applications for rapidly progressive DDEC that lack effective systemic therapy options at present.

Dedifferentiated cancer, Undifferentiated cancer, Drug repurposing screen, 3D spheroid models, Cardiac glycosides

Can protein intake be increased using whole foods in cancer patients post-treatment?

Ravneet Kaur, Caroline Richard, Quincy Chu, Brock Debenham, Wendy Wismer, Vera Mazurak

Poster

Background: Cancer patients experience significant weight and muscle loss during chemotherapy, which negatively impacts their ability to tolerate treatment and leads to dose reductions, decreased survival, and quality of life. To prevent further weight and muscle loss and improve quality of life once treatment is over, stimulating muscle protein anabolism is imperative. However, there is limited published evidence on how to restore nutritional status in cancer patients post treatment. Eggs are a preferred food choice of cancer patients and contain many muscle building nutrients.

Objective: The objective of this study is to determine whether an egg-based dietary intervention is effective in enabling people who have completed chemotherapy to achieve adequate intake of high-quality protein, maintain weight and muscle mass, immune function, and food related quality of life.

Methods: This randomised controlled trial of an 8-week nutritional intervention is enrolling Head and Neck and Lung Cancer patients who have completed their first line platin-based chemotherapy treatment at Cross Cancer Institute, as platin-based chemotherapies are known to evoke muscle and weight loss. Participants will be randomly assigned to either the egg intervention arm (consume at least two eggs daily as part of their usual diet) or to the control arm (usual diet), n=45/group. Body weight, calf circumference, bioelectrical impedance, dietary intake, physical performance, nutritional and immune function markers, quality of life and nutritional status assessment questionnaires will be collected from all patients at baseline, at end of weeks 4 and 8. These assessments will be used to compare nutritional status between groups and longitudinally as a function of egg intake.

Significance: The data obtained from this research in a post-treatment population will provide new information to support guidelines for cancer patients during recovery period and will be beneficial in understanding the dietary requirements of this understudied population.

Project Status: Recruiting(Ethics ID: HREBA.CC-22-0348)

Cancer, Chemotherapy, Muscle loss, Nutrition, whole foods

N-MYRISTOYLATION INHIBITION SHUTS DOWN MITOCHONDRIAL COMPLEX I PROTEIN LEVELS, ACTIVITY, AND CANCER CELL MIGRATION/INVASION

Rony Pain, Erwan Beauchamp, Morris Kostiuk, Heather Mast, Jay Gamma, Eman W. Moussa, Olivier Julien, Hélène Lemieux, and Luc G. Berthiaume

Poster and presentation

In mammals, two N-myristoyltransferases (NMT1 and NMT2) catalyze the addition of the fatty acid myristate ('myristoylation') to >200 different proteins to regulate protein-membrane binding/signal transduction. We demonstrated that the pan-NMT inhibitor PCLX-001 specifically kills hematological cancer cells while sparing normal cells. PCLX-001 treatment results in degradation of otherwise myristoylated proteins by a specific N-degron. To understand NMT substrate specificity and explore PCLX-001's mechanisms of action, we used differential mass spectrometry proteomics of NMT1 and NMT2 CRISPR/Cas9 HAP1 knockout cells and found that the main effect of NMT1 KO was the preferential reduction of numerous respiratory complex proteins instead of signal transducing proteins. The myristoylated mitochondrial complex I assembly factor NDUFAF4 was the most downregulated NMT1 substrate.

While the loss of NMT2, reduced levels of NMT1, and the dependence of cancer cells on NMT1 for survival explained the sensitivity of numerous cancer cells to PCLX-001, it was not a fit for all cancer cells. We generated a myristoylation inhibitor sensitivity signature made of 91 genes, MISS-91, using gene-set enrichment analyses. MISS-91 predicted the sensitivity of numerous solid tumours to PCLX-001 including colon, lung, ovarian, and breast cancers, with the aggressive triple negative breast cancer (TNBC) subtype as the most sensitive.

Since oxidative phosphorylation (OXPHOS) is required for metastasis, we hypothesized that PCLX-001 could inhibit cell migration/invasion by abolishing mitochondrial respiration in MDA-MB-231 TNBC cells. We demonstrate that PCLX-001 promotes the mis-assembly and degradation of mitochondrial complex I, as confirmed by western blot and in-gel activity assays, leading to the loss of respiration. Wound migration and transwell invasion assays showed that PCLX-001 significantly decreased both migration and invasion in a dose-dependent manner. By inhibiting mitochondrial respiration, PCLX-001 may not only inhibit migration and invasion in vitro, but also limit the metastasis of cancer cells in vivo, thereby potentially reducing cancer-related mortality.

Myristoylation, Oxidative phosphorylation, Cancer metabolism, mitochondria, cancer cell invasion

Investigating signaling pathways and therapeutic targets for NF1-inactivated high grade serous ovarian carcinoma: a multiomics study.

Rui Zhe Yang, Jiahui Liu, Guihua Zhang, Martin Koebel, DuPreez Smith, Cheng-Han Lee, YangXin Fu

Poster and presentation

Ovarian cancer is the 5th most deadly cancer in Canadian women, and in the most prevalent subtype, high-grade serous ovarian cancer (HGSC), less than one-third of patients survive past 5 years. Treatments for HGSC are limited, and can include complete resection of the ovaries, fallopian tubes, and uterus. Thus, more effective and less invasive treatments for HGSC are urgently needed. One genetic characteristic of HGSC is more common NF1 inactivation compared to other cancers. NF1 codes for the neurofibromin 1 protein that downregulates the activity of Ras, but it is unclear how NF1 affects HGSC development. Therefore, our objectives are 1) to use a multi-omics approach to investigate how NF1 inactivation affects HGSC phenotypes and signaling pathways in vitro and in vivo, and 2) to find small molecule inhibitors that target upregulated pathways in NF1-depleted HGSC.

We hypothesize that NF1 inactivation can upregulate the pathways related to Ras, increasing cancer cell proliferation and survival. To test this hypothesis, I knocked out NF1 using CRISPR-Cas9 and knocked down NF1 using shRNA in two ovarian cancer cell lines. We generated cell line-derived xenografts (CDX) in mice from NF1-knockout and wildtype NF1 cells and found that phospho-FGFR3 has higher phosphorylation levels in NF1-knockout tumors than in wildtype tumors (n=2). MEK1/2 and ERK1/2 phosphorylation also tend to be higher in NF1-knockout cells compared to wildtype NF1 cells (n=4). NF1 depletion may activate the Ras pathway both upstream and downstream of NF1.

RNA sequencing and phospho-protein mass spectrometry will be used to identify and inhibit druggable proteins that are upregulated in NF1-depleted cells compared to wildtype cells. Any small molecule drugs or drug combinations found to be effective in vitro may be moved to in vivo mouse studies, which have the potential to inform future clinical trials for ovarian cancer patients.

Ovarian cancer, transcriptomics, phospho-proteomics, neurofibromin 1, Ras

Augmented Reality-Based Tumor Localization and Visualization for Robot-Assisted Breast Lumpectomy

Sadra Zargarzadeh, August Sieben, Mahdi Tavakoli

Poster

For early-stage breast cancer, treatment options include mastectomy or lumpectomy. Lumpectomy is often preferred for cosmetic reasons, but accurately locating and tracking tumors during surgery is challenging, especially for impalpable ones. Augmented reality (AR), a technology superimposing computer-generated visuals onto the real world, has shown potential in improving surgeon awareness and the surgeon's sensorimotor augmentation. Although previous work has shown potential in using AR for breast surgeries, its integration with surgical robots while considering breast deformations remains unexplored. We propose an AR-based system for robot-assisted breast lumpectomy, enhancing tumor localization and visualization.

We use semi-deformable phantom tissue and assume while the tissue deforms in addition to rigid motion (translation and rotation), the tumor only undergoes rigid motion. We assume that the initial position of the tumor is known based on ultrasound image data and use a distance-based interpolation algorithm to estimate the tumor's position as the markers placed on the phantom move during the tissue deformations. The real-time updated tumor position is calculated and superimposed on the real video feed using Unity and displayed in the AR medium, the dVRK surgeon's console.

To analyze the performance of our proposed system, we conduct experiments at three stages. In the first stage, we move the phantom in the surgical workspace and analyze the accuracy of marker detection and overlay on the ECM camera feed. Preliminary results show high accuracy of the overlay on both sides of the stereo ECM video. In the second stage, we validate the interpolation algorithm by comparing predicted and actual lesion position on a deformed phantom. Finally, a mock tumor removal procedure is done to evaluate the effectiveness and usability of the system. Preliminary results show promising accuracy in marker localization in 3D and potential for continued development of an accurate and effective system for lumpectomy guidance.

Augmented reality, surgical robotics, breast lumpectomy

The effect of cancer treatment on the development of cancer-related lymphedema

Samaneh Safarpour, Spencer Gibson

Poster

Lymphatic system has an important role in immune and circulatory systems. It modulates tissue fluid homeostasis, and lymphocyte trafficking to lymphoid organs and onto systemic circulation. After chemotherapy and radiation therapy (RT), lymphatic tissue can progress into unrepairable fibrous tissues blocking lymphatic flow and lymphatic proliferation inhibited by RT hinders lymphatic vessel growth. This clinical manifestation incorporates side-effect of cancer treatment, called secondary lymphedema, described as protein-rich fluid accumulation in interstitial tissue owing to lymphatic system disability to transport lymph fluid to circulatory system. This study investigates effects of chemotherapy and radiotherapy and microenvironmental factors on preventing lymphangiogenesis and decreasing proliferation in lymphedema. Two human dermal lymphatic endothelial cells (HDLECs) and Rat gut lymphatic endothelial cells (RatLECs) were treated with a chemotherapeutic drug, cisplatin (CPT (0-100uM)), and doses of X-ray irradiation (0-30 Gy) in the presence and absence of microenvironmental factors including hydrogen peroxide (H2O2), VEGF-C, starvation, and hypoxia. The human umbilical vein endothelial cells (HUVECs) were employed as a counterpart for blood vascular system. Cell death exclusion assay and colony formation evaluated cell growth and proliferation potential. Senescence-associated beta-galactosidase assay was employed to confirm senescence. HDLECs and Rat LECs were resistant to lower doses of drug and irradiation. They indicated cell death at higher doses and in combination with H2O2 (80% and 100% cell death for Irradiation (30Gy)+H2O2 (500uM) at HDLECs and RatLECs respectively and 60% and 40% cell death for CPT (10uM)+H2O2 (500uM) at HDLECs and RatLECs respectively). Colony numbers were significantly reduced by irradiation as low as 5 Gy and CPT as low as 1uM. Beta-galactosidase kit indicated senescence particularly in treatment with radiation. Cancer treatment can push cells toward senescence or quiescence, dispensable for restraining proliferation in lymphatic cells and lymphedema development. Solution is directing cells towards apoptotic cell death by senolytics or resuming proliferation.

Lymphedema, Chemotherapy, Radiation, Microenvironmental factors, Senescence

Development of nanoparticles for delivery of S4Br, a novel competitive inhibitor of polynucleotide kinase/phosphatase (PNKP), in colorectal cancer therapy

Sara Abd El-Hafeez, Cameron Murray, James Donnelly, Mark Glover, Frederick West, Kristie Baker, Michael Weinfeld, Afsaneh Lavasanifer

Poster

Background: Polynucleotide kinase/phosphatase (PNKP) is a bifunctional DNA repair enzyme which phosphorylates DNA 5'-termini and dephosphorylates DNA 3'-termini making the damaged DNA termini amenable for ligation. PNKP inhibitors (PNKPi) can make cancer cells more sensitive to DNA damage by ionizing radiation or Topoisomerase I inhibitors.

Purpose: Our team has identified S4BR as a novel competitive PNKPi (IC 50= 0.9 µM). We aimed to develop a nanocarriers of S4BR and investigate its potential anticancer in colorectal cancer (CRC).

Methods: Three nano-formulations of S4BR were prepared by dissolving it and either of the following three polymers in DMSO with dropwise addition to distilled water followed by dialysis against water using S4BR: polymer (1:10 w/w) ratio. The polymers used were: poly(ethylene oxide)-poly(benzyl-caprolactone (PEO-PBCL), poly(ethylene oxide)-poly(caprolactone) (PEO-PCL) or poly(ethylene oxide)-polymer(D, L-lactic acid) (PEO-PDLA). The prepared formulations were characterized for the level of encapsulated S4Br using UV/vis spectroscopy at 445 nm, and average diameter using dynamic light scattering (DLS). Cytotoxicity of S4BR was measured in wild type HCT116 (WT HCT116) and its Phosphatase and tensin homolog (PTEN) knock out homolog (HCT116 PTEN -/-) using MTT and colony forming assay.

Results: The different polymeric micelles were all in the nano-size range, not exceeding an average diameter of 137 nm. Highest encapsulation efficiency and loading content of S4BR was achieved in PEO-PBCL micelles (91.18% and 8.78%, respectively). A lower IC 50 of S4BR was observed with HCT16 PTEN -/- (IC 50 = 17.7 µM) compared to WT HCT 116 (IC 50= 50.35 µM) indicating synthetic lethality of S4BR in HCT116 PTEN -/- . Clonogenic survival assay showed HCT116 /PTEN -/- to be more sensitive to S4Br at 12.5 µM while no toxicity was observed in WT HCT116 confirming synthetic lethality of S4Br in PTEN deficient CRC.

Conclusion: The data confirms the anti-cancer activity of S4BR in PTEN negative CRC in line with what is expected from a PNKPi. The data also shows a good potential for PEO-PBCL nanocarriers for solubilization and delivery of S4BR in CRC.

Colorectal cancer, PNKP inhibitors, synthetic lethality

Differential modulation of Cytochrome P450 1A1 expression by Dimethylmonothioarsinic acid (DMMTAV) in vivo and in vitro

Sara R. El-Mahrouk, Mahmoud A. El-Ghiaty, Mohammed A. Alqahtani, Ayman O.S. El-Kadi

Poster

Dimethylmonothioarsinic acid (DMMTAV), a pentavalent arsenic thio-derivative, has been detected in various biological samples, including saliva, hair, and nails, of acute promyelocytic leukemia (APL) patients treated with the chemotherapeutic agent, arsenic trioxide. Despite being a minor metabolite in both humans and animals, DMMTAV exhibits significant toxicity potential, capable of inducing carcinogenic effects. This toxicity is thought to result from arsenic compounds' ability to regulate the activity of the cytochrome P450 1A1 (CYP1A1) enzyme, pivotal in both procarcinogen activation and detoxification. In our study, we examined the impact of DMMTAV on CYP1A1 expression, both in the absence and presence of its well-known inducer, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). C57BL/6 mice were subjected to intraperitoneal injections of DMMTAV at 6 mg/kg, administered alone or concurrently with 15 µg/kg TCDD, for 6 and 24 hours. Similarly, murine Hepa-1c1c7 cells were exposed to varying DMMTAV concentrations (0.5, 1, and 2 µM), with or without 1 nM TCDD, over 6 and 24 hours. DMMTAV significantly inhibited TCDD-induced Cyp1a1 mRNA levels in vivo (only at the 6-hour time point) and in vitro, linked to reduced transcriptional activation of the CYP1A1 regulatory element. Notably, DMMTAV had opposing effects in C57BL/6 mice, enhancing TCDD-induced CYP1A1 protein and activity, while suppressing both in Hepa-1c1c7 cells. Although DMMTAV did not affect Cyp1a1 mRNA stability, it negatively impacted protein stability, resulting in a shorter half-life. Constitutive levels of CYP1A1 mRNA, protein, and catalytic activities in DMMTAV-treated C57BL/6 mice and Hepa-1c1c7 cells showed no significant alterations. Our findings underscore that DMMTAV exposure amplifies procarcinogen-induced CYP1A1 catalytic activity in vivo, potentially leading to excessive procarcinogen activation when co-exposed, raising concerns about potential adverse health effects.

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Dimethylmonothioarsinic acid, Carcinogenic, Toxicity, TCDD, CYP1A1

Tumor-Secreted Nucleosides Promote RbFox1 Degradation and Dedifferentiation in Cardiomyocytes; A Two-Hit Hypothesis with Implications for Cardiotoxicity

Saymon Tejay, Maria Areli Lorenzana Carillo, Joseph Nana, Yongsheng Liu, Yongneng Zhang, Alois Haromy, Yuan Zhao, Edith Pituskin, John Ussher, Evangelos Michelakis, Gopinath Sutendra

Poster

Introduction: It is well-documented that tumor cells can secrete numerous signalling factors that affect distant normal tissues. For example, tumor-secreted inflammatory factors can initiate muscle or fat tissue breakdown into protein or fatty acids, respectively, that can be used to feed the growing tumor, but consequently can severely decrease the weight of cancer patients. What remains incompletely understood is if tumor-secreted factors (TSFs) can initiate a signalling cascade in the heart, rendering cardiomyocytes (the contractile cells of the heart) susceptible to cell death when treated with anti-cancer drugs.

Methods: We utilized clinically relevant xenotransplant cancer mouse models to study the effect of secretions on the myocardium. We utilized serum and breast cancer samples from patients that did and did not develop cardiotoxicity. We isolated primary cardiomyocytes to elucidate our proposed signalling cascade. We generated transgenic cardiomyocyte specific RbFox1 KO mice and assessed heart function with echocardiography after anthracycline treatment.

Results: We show that tumor secreted nucleosides (i.e. inosine), which are precursors for generating DNA in the cell, were significantly increased in the serum of mice with lung cancer and breast cancer patients that developed cardiotoxicity. Mechanistically, we found that tumor secreted inosine activates the A2A receptor on cardiomyocytes initiating a signaling cascade leading to degradation of the RNA splicing factor RbFox1. RbFox1 loss induces the formation of the mitochondrial permeability transition pore and release of cytochrome C (a precursor for cell death), while also promoting cardiomyocyte dedifferentiation and a more receptive chromatin for anti-cancer intercalating agents (which induce the pro apoptotic transcription factor p53). In keeping, RbFox1 deficient mice develop significant cardiotoxicity when treated with low dose intercalating agents, including the commonly used doxorubicin.

Conclusions: This work provides both a potential biomarker (i.e. inosine) and mechanism for susceptibility to our most cardiotoxic anti-cancer drugs including doxorubicin and cisplatin.

Purinergic Signalling, Cardio-oncology, Biomarker

The Influence of Arsenic on Selenium Uptake by Red Blood Cell Anion Exchanger 1/SLC4A1 Variants

Serena Li, Kamran Shekh, Naomi Potter, Emmanuelle Cordat, Elaine M. Leslie

Poster and presentation

Chronic exposure to the carcinogen arsenic (As) is a global health concern affecting millions of people world-wide. Arsenic causes lung, skin, and bladder cancers and is associated with kidney and liver tumours, so toxicity prevention and treatment strategies are greatly needed. Arsenic and the essential nutrient selenium (Se) form the seleno-bis (S-glutathionyl) arsinium ion [(GS)2AsSe]- in red blood cells (RBCs) after uptake of selenite (HSeO3-) by the RBC anion exchanger 1 (AE1/SLC4A1). This results in As and Se RBC retention, slowing the distribution of both elements to peripheral tissues, playing a protective role. The SLC4A1 gene is highly polymorphic and over 25 variants are linked to common hereditary human diseases including Southeast Asian Ovalocytosis (SAO). The mutants are well characterized for Cl-/HCO3- but not Cl-/HSeO3- exchange activity. We hypothesize that inter-individual variability in RBC Se transport pathways could influence how well an individual exposed to As responds to Se supplementation as a means of preventing As-induced cancers. We expressed five naturally-occurring AE1 variants in human embryonic kidney (HEK) 293 cells. AE1-wild-type (WT) transfected HEK293 cells demonstrated HSeO3- uptake with a Km of 27 ± 4 µM, comparable to the Km of 55 ± 18 µM in RBCs, supporting AE1 as the main RBC uptake transporter of HSeO3- and validating HEK293 cells as a model. HSeO3- uptake by HEK293 cells expressing empty vector (EV), AE1-SAO, -Leu687Pro, -His734Arg, -Arg760Gln, and -Pro868Leu, was 0.28, 0.55, 0.69, 0.37, 0.34, and 1.92-fold WT, respectively. With arsenite, HSeO3- uptake by HEK293-EV, AE1-SAO, -Leu687Pro, -His734Arg, -Arg760Gln, and -Pro868Leu, was 0.33, 0.71, 0.77, 0.48, 0.43, and 1.97-fold WT, respectively. These data have implications for the use of Se supplementation in human treatment for As exposure; exposed individuals expressing variants that increase Se accumulation in RBCs will likely benefit more from Se supplementation than those with reduced HSeO3- transport.

arsenic, selenium, toxicology, red blood cells

Investigating the role of FABP7 in glioblastoma bioenergetics

Seth Peyton, Won Shik Choi, Roseline Godbout

Poster

Background: Glioblastoma (GBM) is the most common primary brain tumor in adults. With a recurrence rate of over 90% and a median survival time of 12-15 months, it is also a very deadly cancer. One aspect of GBM that makes it difficult to treat is the presence of glioblastoma stem-like cells (GSCs) that are migratory and therapy-resistant. GSCs have been shown to highly express FABP7, which binds and shuttles polyunsaturated fatty acids (PUFAs) within the cell. FABP7 has also been shown to co-localize with mitochondria in GBM. In addition, because GSCs have been shown to primarily use fatty acids to meet their energetic needs, investigating the contribution of FABP7 to GBM metabolism is an important research direction.

Methods: Our lab has a large bank of glioblastoma cell lines, both established and patient-derived. High Resolution Respirometry with the Oroboros Oxygraph 2k was conducted for both A4-004 (patient-derived) and U251 (established) cell lines, which allowed determination of cellular respiration in real-time. First the cells were treated with etomoxir, a CPT1 inhibitor, and compared against a DMSO control. Subsequently, 2-factorial experiments were conducted with etomoxir, an FABP7 inhibitor, and DMSO controls.

Results: From the first experiment, it was shown that oxygen consumption linked to ATP production was markedly reduced when CPT1 was inhibited, with an increased effect in the patient-derived GSC cell line. The 2-factorial experiments showed that most of the ATP-linked oxygen consumption was due to fatty acid oxidation. It was also found that inhibition of FABP7 reduced the overall energy production in the cells.

Conclusions: These data suggest that fatty acid oxidation and FABP7 play an important role in GBM bioenergetics at the level of the mitochondria. They highlight the importance of pursuing PUFA metabolism for clinical treatment of glioblastoma.

Glioblastoma, metabolism, fatty acids, fatty acid binding protein 7, oxidative phosphorylation

Investigating the effects of β-hydroxybutyrate, short chain fatty acids & the changes in cellular energetics on colorectal cancer DNA repair subtypes

Shahad (Shay) Al-Imarah, John R. Ussher, Kristi Baker

Poster and presentation

Purpose: Colorectal cancer (CRC) is currently the 2nd cause of death among cancer patients in Canada and worldwide. Both dietary habits and genetic mutations are highly affiliated with CRC incidence. The subtype of genetic mutations in CRC tumours is often crucial in predicting prognosis. CRC tumours are primarily classified as having either microsatellite instability (MSI) or chromosomal instability (CIN) with MSI cancers having an overall better prognosis and different reactions to chemotherapy. Moreover, diets are also important contributors to CRC, where WHO had reported in 2020 a rise in CRC cases amidst countries with dominant western diets along with a drop in CRC cases in countries with higher dietary consumptions of fibre. High fibre diets have shown protective traits that have been attributed to different metabolites of the fibers' breakdown by the microbiota into short chain fatty acids (SCFAs). The Baker lab has previously found that CRCs with different mutation subtypes utilise different energetic pathways. The purpose of this project is to investigate the effects of microbial metabolites on CRC metabolism and genomic instability.

Hypothesis: We hypothesise that MSI and CIN CRC subtypes utilise different cellular energetics pathways, which will aid in a better prognosis.

Methods: We exposed cells and CRC organoids of MSI & CIN subtypes to different metabolites (β-hydroxybutyrate) and examined their effects on proliferation, DNA repair and metabolic activities.

Results: Preliminary data have shown differences in responses to low glucose and glutamine conditions among CRC subtypes. Dominantly, the MSI subtype has shown the highest rate of DNA damage in low glucose conditions compared to other subtypes.

Conclusions: These findings suggest a potential relation between cancer metabolism and genetic mutations. This can aid in a better understanding of the differences in prognosis among CIN vs MSI tumours and help develop treatments based on DNA repair downstream pathways.

colorectal cancer, Short chain fatty acids, DNA repair, cancer metabolism

Genomic instability and DNA damage regulate STING activation through characteristic cytosolic DNA structures in colorectal cancer

Shayla Mosley, Courtney Mowat, Kristi Baker

Poster

Cancer genomes are characterized by instability, displaying significant levels of DNA damage and mutation due to defects in DNA repair. In colorectal cancer (CRC), mismatch repair defects define the microsatellite instable (MSI) subtype, with stronger immunogenicity and better prognosis than the more common chromosomally instable (CIN) subtype. The mechanisms behind these differences remain unknown, but their understanding could identify therapeutic strategies to improve CIN CRC prognosis. The cGAS/STING pathway detects foreign DNA in the cytosol, leading to type-I interferon production and induction of innate and adaptive immune responses. In cancer cells, STING also recognizes self cytosolic DNA (cyDNA) in the form of micronuclei or diffuse fragments generated through endogenous or treatment-induced DNA damage. Our lab has previously identified STING as a key player in the increased cytotoxic T-cell infiltration characteristic of MSI CRCs. We aim to characterize the cyDNA present in different CRC subtypes, and understand how these characteristics differentially affect anti-tumor immunity. Using MC38 CRC cells with mutations in Mlh1 (modeling MSI) or Kras or Rad51 (CIN), we found equal concentrations of MSI cyDNA more strongly activate STING, and thereby cytotoxic T-cells, than CIN cyDNA. CyDNA from CRC patient organoids show similar results. Radiation or 5-fluorouracil treatments also increase cyDNA stimulatory capacity. Sequencing identified patterns prevalent in more stimulatory cyDNAs, including microsatellites, which we observe enhance STING and cytotoxic T-cell activation through improved cGAS binding. Additionally, we observe increased dendritic cell and cytotoxic T-cell activation in CIN tumors exposed to microsatellites. Finally, micronuclei from MSI CRCs also lead to stronger STING activation than from CIN CRCs, and we will evaluate micronuclei composition to identify underlying mechanisms. This project will further our understanding of immune differences between MSI and CIN CRCs, allowing for future development of therapeutic STING agonists to improve anti-tumor immune activation in CIN CRC patients.

Genomic instability, innate immunity, colorectal cancer

The Role of Inflammation and Adipokines in Chemotherapy Induced Adipose Tissue Wasting in a Pre-clinical Model of Colorectal Cancer

Stanley Woo, Abha Dunichand-Hoedl, Catherine Field, Vera Mazurak

Poster

Background: Colorectal cancer (CRC) patients undergoing chemotherapy often lose both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). However, SAT follows a different pattern of loss compared to VAT, and is more predictive of mortality. Dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may prevent chemotherapy-associated losses of adipose tissue, which is poorly understood. The aim of this study was to assess levels of proinflammatory cytokines, adipokines, and lipogenic/lipolytic proteins within VAT and SAT using a pre-clinical model of CRC treatment with a EPA+DHA containing diet.

Methods: Female Fischer 344 rats were implanted with the Ward colon tumour for two weeks before receiving irinotecan and 5-fluorouracil (50 mg/kg body weight). Rats were either fed a control diet with the macronutrient composition of a western diet throughout the study, or switched to a EPA+DHA enriched diet at the start of chemotherapy. Healthy animals were also fed the control diet, but did not receive tumour or chemotherapy. Rats were killed and VAT and SAT were collected at 4 and 8 days following chemotherapy. Adiponectin, leptin, IFN-γ, IL-1β, IL-6, CXCL1, TNF-α, and IL-10 concentrations were measured with a multiplex assay. ATP citrate lyase, hormone sensitive lipase, adipose triglyceride lipase, and IkB kinase levels were measured with Western blot.

Results and Conclusion: CXCL1 was significantly higher in VAT and SAT 8 days following chemotherapy compared to healthy reference groups. Concentrations in SAT of control diet rats approximately double the CXCL1 levels in VAT from rats of both diets and SAT of EPA+DHA diet rats. Chemotherapy induced 60-70% higher levels of ATP citrate lyase in VAT 4 days following chemotherapy compared to healthy controls. These findings are congruent with different patterns of loss of VAT and SAT observed in cancer patients and suggest CXCL1 as a mechanism of chemotherapy-associated adipose tissue loss.

metabolism, inflammation, lipids, adipose, wasting

Genetic alterations induced by sub-lethal apoptosis

Tomás Gutiérrez, John Maringa Githaka and Ing Swie Goping

Poster

Defective apoptosis is a well-established hallmark of cancer and is an important step in many therapeutic approaches. Typically apoptosis is viewed as an irreversible process regulated by the delicate interplay between pro- and anti-apoptotic proteins, culminating in fatal DNA fragmentation and cell death. Traditionally, apoptosis has been considered a tumor-suppressive mechanism. However, recent studies have challenged this paradigm by highlighting sub-lethal apoptosis as a potentially oncogenic driver, capable of enhancing cancer cell aggressiveness in a heritable and persistent manner. The genomic alterations that presumably drive these phenotype remain unexplored.

In this study, we investigate whether sub-lethal apoptosis induces mutagenesis, contributing to the observed increase in cancer cell aggressiveness. We employed MCF7 breast cancer cells with overexpressed pro-apoptotic protein BIK as a model for sub-lethal apoptosis. Despite its role in promoting cell death, BIK was found to be associated with poor prognosis in breast cancer and increased MCF7 cell aggressiveness. We aimed to assess mutagenesis through whole genome sequencing, including the quantification of mutations, mutational hotspots and signatures, and clonal populations.

Surprisingly, BIK-induced sub-lethal apoptosis did not significantly alter the number, type, or pattern of genomic mutations. These findings challenge the conventional view of sub-lethal apoptosis as a driver of genomic mutations in cancer cells and suggest that an alternative mechanism underlies the observed increase in aggressiveness.

Sub-lethal apoptosis, breast cancer, mutagenesis, whole-genome sequencing

The Influence of ABCC1 Single Nucleotide Polymorphisms on the Cellular Export of the Human Carcinogen Arsenic

William Li, Yingze Ma, Janet R. Zhou, Elaine M. Leslie

Poster

Arsenic is a proven human carcinogen causing cancers of the skin, bladder, and lungs. Arsenic is a global health concern because 92 to 220 million people worldwide are exposed to unacceptable levels of it (>10 ppb) in their drinking water. The ATP-Binding Cassette (ABC) transporter Multidrug Resistance Protein 1 (MRP1 or ABCC1) is known for its role in conferring multidrug resistance to cancer cells via the efflux of chemotherapeutic agents. In addition, ABCC1 effluxes conjugated organic anions, including arsenic glutathione conjugates out of cells, thus establishing a role for ABCC1 in cellular arsenic defense. ABCC1 is highly polymorphic, and while naturally occurring variants have been characterized for their transport of physiological conjugated organic anions such as leukotriene C4 (LTC4), the influence of genetic variability on arsenic metabolite transport has yet to be characterized. We hypothesize that 13 selected single nucleotide polymorphisms (SNPs) of ABCC1 would have decreased transport of arsenic triglutathione [As(GS)3]. These ABCC1 SNPs were transiently expressed in human embryonic kidney (HEK) 293 cells, and the transport of radiolabeled As(GS)3 by plasma membrane enriched fractions was measured. An immunoblot showed that there was no difference in protein levels between the wild-type (WT) transporter and the variants. The findings suggested R230Q, R433S, and A989T-ABCC1 exhibited at least a two-fold decrease in transport of As(GS)3 relative to WT-ABCC1, which will be further characterized by kinetic analyses. Consistent with As(GS)3 transport activity, R433S-ABCC1 had a >2-fold decrease in the transport of LTC4. In contrast, R230Q and A989T-ABCC1 LTC4 activity was similar to WT, suggesting non-identical As(GS)3 and LTC4 binding sites. Individuals who carry ABCC1 SNPs with reduced As(GS)3 transport activity may have increased susceptibility to arsenic-induced disease.

Arsenic, ABCC1, SNPs, Transport, Vesicles

Blocking the autotoxin-LPA-inflammatory cycle changes the breast tumor microenvironment and inhibited tumor development

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Poster

Autotaxin (ATX) is a secreted enzyme that produces lysophosphatate (LPA), which activates six G protein-coupled receptors. This promotes the secretion of inflammatory mediators in a feedforward ATX-LPA-inflammatory cycle, which is one of the drivers of breast cancer cell proliferation and metastasis . However, breast cancer cells express negligible quantities of ATX and they rely on ATX produced by other cells in the tumor microenvironment (TME). This cross-talk among cells in the tumor controls breast cancer progression but this is not well understood. We analyzed the single cell RNA sequencing (scRNA-seq) data of human breast tumors and found that ATX is expressed mainly in endothelial cells and fibroblasts. LPA signaling within breast tumors also depends on the differential expression of LPA receptors. The scRNA-seq data showed that LPAR1 is expressed in fibroblasts, LPAR2 is expressed in cancer cells and T cells, LPAR5 is expressed in myeloid cells, and LPAR6 is expressed in endothelial, cancer cells, fibroblasts, and myeloid cells. Bioinformatics analysis also indicated that ATX expression level is negatively correlated with T cell mediated immune responses and positively correlated with inflammation and signaling pathways of IL-6, LIF, TGFβ, and prolactin in human breast tumors. These results suggest that targeting the ATX-LPA-inflammatory cycle will have a profound impact on multiple cell types in TME. We demonstrated that the ATX inhibitor, IOA-289, decreases breast tumor growth in mice. This was accompanied by decreases in the concentrations of CXCL10, CCL2, and CXCL9 in the plasma and LIF, TGFβ1, TGFβ2, and prolactin in the tumors. Attenuating the ATX-LPA-inflammatory cycle using Infliximab, an antibody against TNF□□ decreases ATX levels in breast tumors and significantly suppresses lung metastasis.

tumor microenvironment, autotaxin, inflammation

Novel Polyethyleneimine Derivatives for Gene Silencing in Cancer

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Poster and presentation