Presenter's Name: Dawson, Allie

Additional Author(s): Shaikh M, Barrett J, Nichols AC

Abstract Title: Investigating Biomarkers of Treatment Resistance in Human Papillomavirus-Related Head and Neck Cancer

Abstract:

Introduction: Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer worldwide. Recently, infection by human papillomavirus (HPV) has caused a rapid rise in HNSCC cases. Although patients with HPV+ HNSCC generally respond well to chemoradiation treatment, a cohort of patients exhibit treatment resistance leaving them more susceptible to tumour recurrence and metastasis. At present, no known molecular drivers to treatment resistance in HPV+ HNSCC have been identified therefore, we have completed genome and transcriptome analyses of a HPV+ HNSCC cohort. With this resource, copy number losses of MACROD2 have been identified in the treatment failure dataset. Thus, we hypothesize that deletion of MACROD2 drives resistance to chemoradiation in HPV+ HNSCC.

Methods: Functional validation in vitro and in vivo will be completed to assess MACROD2 as a candidate gene for treatment resistance. This involved siRNA knockdown and CRISPR knockout of MACROD2 in HPV+ HNSCC cell lines. Functional assays will then be completed in vitro including proliferation, clonogenic, cisplatin-sensitivity, and radiation-sensitivity. To assess chemoresistance in vivo, CRISPR edited, and control cell lines will be injected into mice and weekly cisplatin treatments will be administered at varying doses. To assess radioresistance in vivo, CRISPR edited cells will be irradiated and injection into mice. Following both treatments, tumour growth rates will be compared between control and altered models.

Results: To date, preliminary siRNA screens have suggested MACROD2 as a potentially significant gene related to chemoradiation resistance in HNSCC. Further, MACROD2 knockouts have been generated in HPV+ HNSCC cell lines and validation efforts to confirm knockout is currently underway. Functional assays in vitro and in vivo will be completed at which point we expect to see differences between control and CRISPR-edited cell lines.

Discussion: These findings will provide a better understanding of the molecular basis of treatment resistance in HPV+ HNSCC and may contribute to patient management stratification measures for all HNSCC patients.

POSTER PRESENTATIONS 2
2D: CANCER BIOLOGY

Presenter's Name: Houpt, Jacob A.

Additional Author(s): Wang H, Liu E, Han Y, Ling C, Cecchini MJ, Zhang Q

Abstract Title: Determination of Ki-67 Indices in Neuroendocrine Tumours of the Gastrointestinal Tract: A Comparison of Manual and Digital Methods at London Health Sciences Centre

Abstract:

Introduction: Since its discovery, Ki-67 has remained a ubiquitous means of assessing proliferation and aggressiveness of neoplasms, representing an invaluable staple in pathology for diagnoses, prognoses, and informing treatment. One example of this application is the use of Ki-67 index in the grading of gastrointestinal neuroendocrine tumours (GI NETs). However, variability persists among pathologists in methods used to determine Ki-67 index and in the indices themselves, with cell detection software poised to improve efficiency and consensus across observers. This study compares manual and digital determination of Ki-67 indices by LHSC pathologists, levels of confidence in each method, and time expended to determine which methods can best balance accuracy with time required.

Methods: 12 GI NETs diagnosed at LHSC in 2021 were retrieved, associated with various sites along the GI tract, patient demographics, and grades at time of diagnoses. 9 pathologists were provided with H&E and Ki-67 slides/images and asked to evaluate the Ki-67 indices via manual estimation, web-based image analysis using cellular segmentation (AI4Path with Cellpose), and software-based image analysis using cell classification (QuPath). Responses and demographic information for participants were collected via Qualtrics surveys and a minimum 1-week wash-out period was used to mitigate the possibility of recalling previous responses.

Results: AI4Path was associated with lower variability across pathologists when compared to manual estimation and QuPath once abnormal hotspot selection was accounted for. It was also the method with the greatest time expended per case for most participants, except for GI NETs with Ki-67 index values close to grade thresholds (3% and 20%). AI4Path and QuPath achieved greater levels of grading consensus when compared to manual methods, highlighting the utility of digital pathology methods when applied to near-borderline cases of GI NET Ki-67 indices.

Discussion: Our findings indicate the potential for digital pathology to improve efficiency and accuracy in the evaluation of Ki-67 indices, but that challenges persist in its practical implementation in the form of ease of use and time required to scan whole-slide images. While less applicable in cases of very low or high Ki-67 index GI NETs (which had grading consensus across all methods), both AI4Path and QuPath methods may have utility in more efficiently calculating Ki-67 indices in borderline cases.

Presenter's Name: Lewis, Natalie

Additional Author(s): Greasley A, Abu Omar AA, Zheng X

Abstract Title: The Role of Circular RNA in Colon Cancer Cells in Response to Chemotherapeutics

Abstract:

Introduction: Colon Cancer is a disease with both high incidence and mortality rates. It is commonly treated with the chemotherapeutic drug 5-Fluorouracil (5-FU), among other interventions. However, the development of resistance to 5-FU has emerged as a major problem interfering with the ability to successfully treat this disease. Circular RNAs (circRNAs), a type of non-coding RNA, have been increasingly studied in the context of cancers, and some have been shown to play a role in drug resistance. CircRNA PNN (circPNN), has previously been shown to be upregulated in colon cancer patients, however its involvement in chemotherapy-induced cell death remains unknown. This study aims to demonstrate the effect of circPNN on the response of colon cancer cells to chemotherapeutic treatment with 5-FU.

Methods: To understand how circPNN expression changes in response to chemotherapy, the expression profile of circPNN is being determined using quantitative real-time PCR (q-PCR). We treated HT29 colon cancer cells with increasing doses of 5FU, and circPNN expression will be detected at different timepoints after treatment. To explore the effect of circPNN on the response of colon cancer cells to treatment with 5-FU, we designed small interfering RNAs (siRNAs) to knockdown circPNN. We transfected HT29 cells with the siRNAs and cell death upon treatment with 5-FU will be compared between normal colon cancer cells and circPNN-knockdown cells. Cell death will be detected using an MTT assay, an LDH assay, and dynamically with an Incucyte system.

Results: Preliminary results indicate that circPNN is upregulated by 5-FU. We expect that knocking down this circRNA will enhance the sensitivity of colon cancer cells to this chemotherapeutic agent. Increased cell death in response to treatment is expected to be seen in the colon cancer cells that have been transfected with the siRNA designed to knock down circPNN.

Discussion: This study will elucidate the impact of circPNN on the sensitivity of colon cancer cells to the anti-cancer drug 5-FU. It may provide a foundation for future investigations of methods to improve the chemotherapeutic treatment of colon cancer. Additionally, the results from this study will add to the limited knowledge of the functions of circRNA in drug resistance.

POSTER PRESENTATIONS 2
2D: CANCER BIOLOGY

Presenter's Name: Lui, Ryan

Additional Author(s): Roes M, Dick FA

Abstract Title: The Role of TBX18 on Resistance Development to Enzalutamide in Prostate Cancer

Abstract:

Neuroendocrine prostate cancer (NEPC) is a lethal subtype of prostate cancer that is characterized by low or absent androgen receptor (AR) expression, independence of AR signalling, and gain of a neuroendocrine phenotype. Transdifferentiation from prostate adenocarcinoma to NEPC confers resistance towards next-generation androgen receptor therapies such as enzalutamide (EZ). Our lab has demonstrated that RB-p53 deficiency increases the propensity for LNCaP cells to acquire resistance to EZ, indicating underlying genetic mechanisms that misregulate stemness and cell differentiation pathways. Subsequently, a CRISPR knockout screen was performed on LNCaP cells to determine if the deletion of other genes would also confer EZ resistance. Amongst other genes, TBX18, a transcriptional repressor, was overrepresented. This study aims to investigate the role of TBX18 on EZ resistance in prostate cancer and to elucidate the mechanism of transdifferentiation to NEPC. We hypothesize that a gene knockout of the transcription factor, TBX18, will result in an increased propensity to acquire resistance to EZ in prostate cancer. We performed a CRISPR-Cas9 knockout of TBX18 in a LNCaP cell line and have achieved a population of LNCaP cells with decreased expression of TBX18, confirmed by western blot. Presently, we are examining immediate EZ resistance using an alamarBlue cell viability assay and developed EZ resistance over time through a colony forming assay. The molecular basis of EZ resistance will be investigated by comparing gene expression changes between TBX18 knockout cells and control cells. qPCR will be used to detect expression of known genes that regulate stem cell plasticity such as SOX2 and genes that are known biomarkers of neuroendocrine tumours such as SYP, CHGA, and NSE. Our acquired population of cells likely contains a mixture of partial and full TBX18 knockout cells. Thus, we also seek to isolate and clonally expand a population of cells with full knockout of TBX18 through a limiting dilution assay, which will give greater validity to our findings. Ultimately, the development of resistance to AR inhibitors is a major cause of morbidity and mortality in prostate cancer patients. This study may lead to the discovery of a new biomarker to detect NEPC and identify target pathways for the development of novel therapeutics.

Presenter's Name: Morin, Monique

Additional Author(s): Brackstone M, Burton JP

Abstract Title: Re-setting the breast microbiome to lower inflammation and risk of cancer

Abstract:

Introduction: As of 2021, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death worldwide. Despite major advances in understanding and treating breast cancer, there are still a multitude of "environmental factors" that remain to be understood. One factor of interest is the microbiome of breast tissue. The population of bacteria found in breast cancer tissue has been shown to be more pathogenic than bacteria found in healthy control tissue. The pathogenic bacteria species were known to induce DNA damage and promote inflammation, two hallmarks of cancer. Assuming that there is a link between the pathogenic bacteria and cancer progression and/or induction, we are interested in exploring the possibility of reducing the inflammatory microbiota and consequently reducing the risk of breast cancer. Probiotics have recently emerged as a promising treatment for inflammation of the breast. Previous studies have found that ingestion of probiotic lactobacilli is able to treat inflammatory breast disease in women. We will investigate whether women at high-risk of breast cancer also have inflammatory microbiomes and if we are able to re-set their microbiomes using probiotic lactobacilli.

Hypothesis: We hypothesize that the breast microbiome of women at risk of cancer has the same profile as women with cancer, and that oral administration of probiotic lactobacilli can re-set this to one found in healthy women. We also hypothesize that the probiotic administration will be able to lower inflammatory cytokine levels.

Methods: 20 women at high risk of developing breast cancer and 20 healthy control women will be randomized to either taking the oral probiotic lactobacilli or placebo for 90 days. Participants will have urine, blood, and breast samples taken at day 0, day 90, and day 120. 16sRNA Illumina Miseq of the breast fine needle aspirate samples will be used to identify bacteria populations. Blood plasma will be used to measure the levels of cytokines using Luminex immunoassay kits.

Significance: This study could identify if an aberrant microbiota is linked to women at high-risk of breast cancer and can be reverted back to a healthy microbiome. If such a link exists, we can further follow women at high-risk of breast cancer and see if probiotics can delay the onset of breast cancer. This study will also help us identify inflammatory markers associated with an aberrant microbiota and if probiotics are able to reduce these levels.

POSTER PRESENTATIONS 2
2D: CANCER BIOLOGY

Presenter's Name: Pham, Michelle

Additional Author(s): Lin SXJ, Cecchini MJ

Abstract Title: Tumor clustering index as a novel measure of tumor cell organization in non-small cell lung cancer

Abstract:

Introduction: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths in Canadians. Most NSCLCs can be subtyped into two broad categories: adenocarcinoma or squamous cell carcinoma. NSCLCs present with a number of morphologically distinct patterns that are taken into account when grading these tumors. The clustering of the tumor can be defined by a clustering value (CV) that ranges from absolute clustering (CV = 0) to random clustering (CV = 100) to uniform distribution (CV = 215). In this study, we report the spatial arrangement of adenocarcinoma and squamous cell carcinoma samples from diagnostic slides to better understand the variability in tumor cell organization.

Methods: 40 cases of lung adenocarcinoma and 40 cases of squamous cell carcinoma were randomly drawn from the Cancer Genome Atlas (TCGA) dataset. Cell detection and object classifier were performed using QuPath's built in tools to both detect and classify cells as tumor and non-tumour. Delauney clustering analysis was conducted on each case to determine the mean minimum distance between neighboring tumor cells with a threshold distance of 15 μm. CVs were calculated for each case based on minimum Delaunay distance normalized by the number of tumor cells in a given area. The cell densities of both subtypes and the grade of the tumor in squamous cell carcinoma cases were confirmed by a thoracic pathologist and correlated with CVs.

Results: Adenocarcinoma cases had a mean CV of 90.50 ± 17 compared to squamous cell carcinoma cases with a mean CV of 76.84 ± 26. Adenocarcinoma mean CV was closer to random clustering of tumor cells whereas squamous cell carcinoma had a lower mean CV ($p=0.007$), reflecting more cell clustering. Higher CVs in both adenocarcinoma and squamous cell carcinoma were associated with greater tumor density measurements. In squamous cell carcinoma, there was a trend toward higher CVs in cases with high-grade differentiation, but no significant association.

Discussion: The increased clustering in squamous cell carcinoma cases aligns with the morphology of the tumor that is typically composed of sheets and nests of polygonal cells tightly adhered through cellular junctions. This is in contrast to adenocarcinomas that form variably complex glandular structures that are less tightly clustered together. Future work aims to correlate CVs against NSCLC cases in larger datasets at varying threshold distances as a potential novel prognostic feature.

Presenter's Name: Ratushny, Liam

Additional Author(s): Howlett C, Dick FA

Abstract Title: Investigation of molecular mechanisms of the cancer cell cycle and their impact on treatment resistance and disease progression

Abstract:

Introduction: With breast cancer the world's most prevalent cancer, treating metastases of this disease is a significant clinical issue. This is difficult since mechanisms that allow tumor cells to survive and proliferate at secondary sites are unclear. Experimental models have confirmed disseminated tumor cells (DTCs) can enter dormancy and resist chemotherapy. This dormancy implies a counter-intuitive growth arrest to enhance survival. These findings suggest dormant DTCs are critical to tumor recurrence in breast cancer.

Impaired DREAM assembly in ovarian cancer cell lines compromises cell viability under dormant conditions. The DREAM complex contains DP, Rb-like protein (p130, p107), E2F, and MuvB. This multi-protein complex represses gene promoters and maintains cellular quiescence in dormancy. DYRK1A phosphorylates the MuvB core that binds to p130/p107 to mediate DREAM assembly. DYRK1A deletion/inhibition or p130 deletion causes a loss of cell cycle dependent gene repression, loss of cellular quiescence, and cell death in dormant culture conditions.

I hypothesize the loss of DREAM assembly in breast cancer cells will impair survival of DTCs and reduce development of secondary tumors.

Methods: To test if DREAM deficiency disrupts dormancy and metastasis, a xenograft mouse model will be used to recapitulate metastases. Mice will receive tail vein injections of p130-KO, DYRK1A-KO, or unmodified breast cancer cells. One control group will receive CX-4945 treatments, a kinase inhibitor with DYRK1A inhibitory capabilities. Investigating DYRK1A inhibition/loss and comparing metastatic spread will offer valuable insights into the role of DYRK1A inhibitors as a potential treatment to minimize metastatic spread.

Results: Preliminary xenograft experiments identified that mice that received xenografts of p130-KO breast cancer cells exhibited lower rates of metastasis as well as smaller metastatic nodules when compared to those that received unmodified cells.

Discussion: Identifying a potential mechanism through which circulating breast cancer cells can enter a dormant state to enhance survival, is an exciting new therapeutic target. These findings will elucidate molecular mechanisms of metastasis and potentially identify novel therapeutic targets that can limit metastatic spread and improve overall patient outcomes.

POSTER PRESENTATIONS 2
2D: CANCER BIOLOGY

Presenter's Name: Roes, Michael

Additional Author(s): Dick FA

Abstract Title: HOXA9 promotes enzalutamide resistance in RB-p53 deficient prostate cancer

Abstract:

Introduction: Castration resistant prostate cancer (CRPC) cells can acquire resistance to the anti-androgen enzalutamide (EZ) by switching lineages from an adenocarcinoma to a neuroendocrine (NE) cell type that no longer requires androgen signaling for growth. Cancer genomic studies identified loss of the retinoblastoma (RB1) gene as a defining feature of these cells. Mutations in RB1 cause epigenetic instability, accompanied by up-regulation of pluripotency factors. While mechanistic insight into epithelial de-differentiation is limited, RB1 loss was identified as the most significant factor in determining poor survival and therapy resistance for CRPC patients. In this study, I hypothesize that RB1 loss misregulates stemness and differentiation pathways that result in an increased propensity to transdifferentiate and acquire EZ resistance in prostate cancer.

Methods and Results: A CRISPR knockout screen was performed in prostatic adenocarcinoma LNCaP cells to identify gene mutations that confer resistance or sensitivity to EZ. Gene ontology (GO) analysis of de-enriched genes following EZ treatment identified genes related to stem cell differentiation, such as HOXA9, highlighting the importance of a stemness phenotype for viability in EZ. RB1 mutation was highly enriched following treatment, confirming that RB loss promotes EZ resistance. Since combined RB-p53 loss is known to promote NE transdifferentiation, RB-p53 double knockout (DKO) LNCaP cells were generated. DKO cells display no difference in IC50 values following acute EZ treatment, but form distinct colonies following chronic treatment, compared with LNCaP control (CTL) cells. RNA-sequencing of DKO and CTL cells, followed by GO analysis, revealed differentially expressed genes related to neuronal processes and cell differentiation. The stemness factor HOXA9 was also significantly upregulated in DKO cells. Intriguingly, in clinical EZ-resistant prostate tumour samples, HOXA9 mRNA is positively correlated with NE features. CTL and DKO cells engineered to overexpress HOXA9 have increased IC50 values following acute EZ treatment, compared with both parental lines. HOXA9 over-expression in DKO cells also caused the formation of significantly more colonies following chronic EZ treatment, compared with parentals.

Discussion: Overall, these results suggest that HOXA9 promotes EZ resistance in prostate cancer. Furthermore, HOXA9 inhibition may be of therapeutic benefit for treating EZ-resistant CRPC.

Presenter's Name: Ryan, Sarah Belle

Additional Author(s): Zeng P, Barrett J, Nichols AC

Abstract Title: Exploring the Effects of Naporafenib on MAPK Pathway Proteins and Immune Checkpoint Inhibitor Levels in Anaplastic Thyroid Cancer Cells Lines

Abstract:

Introduction: Anaplastic thyroid cancer (ATC) is an undifferentiated malignancy that accounts for less than 2 percent of total thyroid cancer diagnoses. ATC is one of the most lethal human malignancies, with no known treatments with long-term effectiveness. There are approved targeted treatments if specific mutations are present, but resistance usually develops in less than a year. The MAPK pathway is often mutated in ATC, including BRAF and RAS. Current treatments for BRAF mutant ATC include first generation BRAF inhibitors, which can be used with MEK inhibitors such as Trametinib. First generation BRAF inhibitors can cause paradoxical MAPK activation in RAS mutant ATC. Second generation BRAF inhibitors, such as Naporafenib, have been developed but are not currently approved for clinical use in ATC. This project aims to determine the mechanisms by which Naporafenib inhibits the growth of ATC cell lines, how resistance to Naporafenib develops in ATC cell lines, and if Naporafenib can overcome resistance to first generation BRAF inhibitors. We hypothesize that unlike first generation BRAF inhibitors, Naporafenib will be effective against BRAF and RAS mutant ATC. We further hypothesize that Naporafenib can overcome resistance to first generation BRAF inhibitors.

Methods: UHTH7, Ash 3, and SW1736 ATC cell lines were cultured. Cells were incubated with DMSO, 5 μM of Naporafenib, 10 nM of Trametinib or 5 μM Naporafenib and 10 nM Trametinib combined for 24 and 48 hours. Western blots were performed for MAPK proteins and immune checkpoint inhibitor PDL1.
Results: After incubating with DMSO, Naporafenib, Trametinib or the combination of Naporafenib and Trametinib for 24 hours, a decrease in PDL1 was apparent in Ash 3 cells treated with Naproafenib, while an increase in PDL1 was seen in UHTH7 cells treated with Naporafenib. No differences in PDL1 were observed in SW1736 cells at 24 hours. After 48 hours a decrease in PDL1 was seen in all Naporafenib treated cell lines.

Discussion: Exploring the effects of Naporafenib on MAPK and immune checkpoint inhibitor levels in ATC will provide a deeper understanding of a targeted therapy with potential to treat ATC. Preliminary work suggests that Naporafenib may impact PDL1 levels, indicating a combination of targeted therapy and immune therapy should also be investigated in ATC. Next steps include the creation of LXH 254 resistant ATC cell lines and the optimization of LXH 254 concentration and incubation time.

POSTER PRESENTATIONS 2
2D: CANCER BIOLOGY

Presenter's Name: Zakirova, Komila

Additional Author(s): Passos DT, Dick FA

Abstract Title: Identification of molecular mechanisms of spheroid dormancy in epithelial ovarian cancer

Abstract:

Metastatic dissemination of cancer cells is the major contributor to the mortality in epithelial ovarian cancer (EOC). Advanced stage EOC is characterized by the accumulation of ascites where these cells aggregate to form multicellular clusters, or spheroids. EOC spheroids become dormant by exiting cell cycle and as a result insensitive to chemotherapy. The persistence of drug-resistant cancer cells remains a major challenge in successful treatment of EOC and highlights the importance of elucidating the molecular mechanisms required for the formation and viability of spheroids.

To identify genes and pathways that contribute to cellular dormancy, known negative growth regulators have been previously disrupted in a panel of EOC cell lines. DYRK1A-dependent assembly of the DREAM repressor complex was identified as a key mediator of growth arrest in spheroid cells. Loss of DYRK1A activity, via genetic deletion or chemical inhibition in EOC cells, hindered spheroid formation in a model system of cellular dormancy. Chemical inhibition of DYRK1A also resulted in improved sensitivity to carboplatin, suggesting it may have therapeutic potential in EOC treatment. Further investigation is needed to establish whether the phenotype of DYRK1A deficiency is recapitulated in vivo. The role of other downstream mediators of spheroid dormancy and their relationship to DYRK1A function is yet to be established. Overall, this study seeks to systematically identify downstream targets of DYRK1A activity and establish their functional significance in a spheroid model system. Additionally, through in vivo experiments, it aims to assess the therapeutic potential of DYRK1A inactivation. Preventing the process of chemo-resistant spheroid metastasis is fundamental for improving the outcomes of EOC patients.