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.
**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.
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.

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

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

Additional Author(s): Brackstone M, Burton JP

Presenter’s Name: Morin, Monique
**Abstract:** Investigation of molecular mechanisms of the cancer cell cycle and their impact on treatment resistance and disease progression

**Introduction:** With breast cancer the world’s most prevalent cancer, treating metastases of this disease is a significant clinical issue. Understanding the molecular mechanisms behind metastasis can provide new therapeutic targets. 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.

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**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 both parental lines. 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 parents.

**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:** Zakirova, Komila

**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.