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CANCER RESEARCH SYMPOSIUM & STUDENT POSTER COMPETITION

19
APRIL
2024

UI Health | UIC
UNIVERSITY OF ILLINOIS CANCER CENTER

Poster Competition Abstracts

Poster 1

Prarthana Prashanth

Impact of Nutrient Availability on Cellular Sensitivity to Rigosertib

BACKGROUND, PURPOSE, or OBJECTIVES: While several cancer drugs tested in the lab under RPMI media show promising results, many of these drugs have been failing in clinical trials. Studying this further, researchers realized that RPMI is not an accurate representation of human physiological conditions. Instead, Human Plasma-Like Media (HPLM) media serves as a better representation. The Coloff lab conducted a large screening on 626 drugs/inhibitors comparing their effectiveness in HPLM vs commercial RPMI and found striking differences in cellular sensitivity to Rigosertib, a chemotherapy drug, in the two medias. While Rigosertib has been working in lab tests and mice models, it has not been working in human clinical trials. HYPOTHESIS/AIMS: While lab organisms like mice have uricase, an enzyme that breaks down uric acid allowing it to leave the body, humans do not. Hence, uric acid can build up in humans leading to conditions like hyperuricemia and gout. In fact, many cancer patients display hyperuricemia due to the uric acid released via cell death. Hence, we hypothesize that elevated uric acid levels in cancer patients prevents Rigosertib from killing cancer cells. MATERIALS or METHODS: Four cell lines were primarily used: HCC1806 and SUM149 (breast cancer lines), HeLa cells, and K562 (lymphoblast cells). First, we conducted dose curves using various concentrations of Rigosertib, and observed cancer cell death in RPMI versus HPLM. Next, individual components of HPLM were added back to the media to determine if uric acid could be responsible for allowing cancer cells to survive. Finally, cell-cycle analysis was conducted focusing on cell-cycle arrest, and to confirm findings visually, we performed immunofluorescence imaging. RESULTS: From our experimentation, there is a drastic difference in cell sensitivity to Rigosertib based on the media they are exposed to. Cells treated with Rigosertib in RPMI die almost immediately, while cells treated with Rigosertib in HPLM continue to survive. This effect was observed in breast cancer cells, lung cancer cells, lymphatic cells, and more. When individual components were added back to HPLM media, it was observed that cells treated with Rigosertib continued to survive only when uric acid was added back to the media. The cell cycle analysis also shows that under the influence of Rigosertib alone, most cells are in the G2/M phase indicating cell-cycle arrest, while under Rigosertib and uric acid, most cells are in the G1/S phase indicating cell-proliferation. Finally, the immunofluorescence results visually show that all microtubules are dissolved when exposed to Rigosertib; however, under uric acid in combination with the drug, the cells appear healthy. CONCLUSIONS: From this study, we found that in the presence of uric acid, cancer cells exposed to the drug Rigosertib continue to survive. This is important as uric acid is already known to be elevated in cancer patients, as tumors release uric acid when dying. Hence, this could be one of the reasons Rigosertib has been failing in humans. In the long-run, by putting patients on low-purine diets with less uric acid content (no red meat), Rigosertib may work better in patients.

Poster 2

Sai Santosh Babu Komakula

Role of myeloid cell-derived HMGB1 in the development of hepatocellular carcinoma

Abstract: Background: High mobility group box 1 (HMGB1) is a non-histone chromatin-associated protein involved in the pathogenesis of chronic liver disease including hepatocellular carcinoma (HCC). HMGB1 is expressed in myeloid cells, including conventional dendritic cells (cDC), which play a major role in the tumor microenvironment. However, whether intracellular myeloid cell-derived HMGB1 is involved in HCC is unknown. Hypothesis: We hypothesize that intracellular HMGB1 drives cDC maturation towards LAMP3+ DCs, allowing effective cytotoxic CD8+ T cell responses to reduce HCC. Methods: we analyzed publicly available scRNA-seq datasets from human HCC for the expression of HMGB1 in all subsets of DCs in HCC tumor and non-tumor tissue and in hepatic draining lymph nodes (dLNs). We generated mice with conditional ablation or overexpression of Hmgb1 in myeloid cells (Hmgb1_Mye and Hmgb1KI Mye). To induce HCC, 14-day-old male mice were injected i.p. with diethylnitrosamine (DEN) and were sacrificed at 5, 6, and 8 months. H&E-stained liver sections were used for histopathological analysis. Immune cell populations in liver tumor and non-tumor tissues and dLN were analyzed using flow cytometry. LAMP3+ DCs in tumor and non-tumor tissues were examined using immunohistochemistry and gene expression analysis. Fluorescence-associated cell sorting (FACS) isolated cDCs from tumor and non-tumor tissues were analyzed using RNAseq. Results: mature LAMP3+ DCs increase in human HCC tumor tissue and hepatic dLNs. HMGB1 expression is higher in cDCs, which can be developed to mature LAMP3+ DCs. Hmgb1KI Mye mice are protected from HCC, whereas control mice develop HCC after 8 months, and Hmgb1_Mye mice start developing HCC at 5 months. Macroscopic analysis and H&E staining of the livers from Hmgb1_Mye mice show more tumors and higher tumor volume than control and Hmgb1KI Mye mice. The analysis of Immune cell populations shows that Hmgb1_Mye mice have less mature LAMP3+ DCs in liver and hepatic dLNs than control and Hmgb1KI Mye mice, suggesting less CD8+ T cell activation. In addition, there is enhanced CD8+ T cell apoptosis in the HCC tumor tissue from Hmgb1_Mye mice. RNAseq data analysis of cDCs shows that Hmgb1_Mye mice have reduced maturation and antigen presentation markers compared to control and Hmgb1KI Mye mice. Moreover, Hmgb1_Mye mice have reduced mitochondrial subunits in the cDCs, indicating mitochondrial dysfunction. Conclusion: ablation of myeloid-derived HMGB1 accelerates HCC development in mice. There are fewer LAMP3+ DCs in both tumor and dLN of Hmgb1_Mye mice. Therefore, increasing HMGB1 expression in specific myeloid cell subsets (cDCs) could be a therapeutic approach to protect from HCC.

Poster 3

Jane Miglo

Assessing the effects of ovulation on early events in ovarian cancer development

BACKGROUND/PURPOSE/OBJECTIVES: High grade serous ovarian cancer (HGSOC) is the most prevalent and lethal histotype of ovarian cancer. The fallopian tube is the primary origin of most HGSOCs, supported by evidence from molecular analysis and clinically demonstrated by the protective effect of salpingectomy. Ovulation is a major risk factor for ovarian cancer. Currently, the only means for prevention are through the reduction of lifetime ovulatory events, such as through the use hormonal birth control or surgical removal of the fallopian tubes and ovaries. The molecular mechanisms underlying ovarian cancer initiation are largely unknown. Studying the effects of ovulation on ovarian cancer formation is incredibly challenging as ovulation is a dynamic process that occurs deep in the abdominal cavity and requires invasive procedures to access. Our collaborative team has developed a physiologically accurate menstrual cycle model using the PREDICT Multi-Organ System (MOS), a microfluidic (organ-on-chip) device that enables us to recapitulate the process of ovulation and capture the entirety of ovarian secretions. HYPOTHESIS/AIMS: The aim of this project was to evaluate the transcriptional and phenotypical changes that occur in a pre-cancerous fallopian tube cell model treated with ovarian secretions from three phases of the menstrual cycle: follicular, ovulation, and luteal. We hypothesize that the use of a microfluidic system will yield insight into the mechanisms underlying the initiation of HGSOC in the fallopian tube epithelium (FTE) in response to ovulation. MATERIALS/METHODS: Our lab engineered a preneoplastic murine oviductal epithelial (MOE) cell model that represents the earliest known aberration in HGSOC: Pax2-null secretory cell outgrowth (SCOUT). We surgically isolated mouse ovaries and performed the ovulation protocol on the PREDICT microfluidic platform to collect secretions from the follicular, ovulation, and luteal phases of the menstrual cycle. We performed mRNA sequencing on SCOUT cells treated with ovarian secretions followed by bioinformatic pathway analysis. Additionally, we performed a proliferation assay, western blots, and immunofluorescence microscopy to further evaluate the cellular effects of ovulation on SCOUTs. RESULTS: Utilizing ovulation secretions captured on the platform, we found an upregulation of DNA damage response transcripts in the SCOUT model such as RAD51, BRCA1/2, CDK2, and ATM. We also found an upregulation of transcripts important for ovarian cancer development: RUNX2, CD44, FASN, and FABP4. In the luteal phase, we observed an upregulation of NOTCH1 and STAT2. We also detected a significant increase in proliferation caused by secretions from the luteal phase. CONCLUSIONS: Our findings demonstrate that ovarian secretions induce DNA damage response transcripts and promote proliferation in a preneoplastic FTE cell model. These findings shed light on the phase-dependent mechanisms underlying the initiation of HGSOC in the fallopian tube in response to secretions from the ovary.

Poster 4

Alexa M Gajda

EXPLORING THE THERAPEUTIC EFFECT OF K+ CHANNEL AGONISM ON DISSEMINATED BREAST CANCER

BACKGROUND, PURPOSE, or OBJECTIVES: Metastasis is the leading cause of breast cancer mortality worldwide, and its diagnosis is associated with a dismal 5-year survival rate under 30%. Currently, there are no curative treatments at this stage, and most therapeutic options rely on biochemical interventions while overlooking biophysical vulnerabilities of cancer which can be exploited for therapeutic targeting. Recently, our group identified cancer cell stiffness, controlled by myocardin related transcription factor A (MRTFA)'s impact on actin polymerization, as a trigger for mechanosurveillance. HYPOTHESIS/AIMS: In this study, we also identified several cation transport genes upregulated in MRTFA-overexpressing mouse breast cancer cells, including a highly expressed regulatory subunit of a large conductance voltage-gated and Ca2+ -activated K+ channel (BK), KCNMB1. Because KCNMB1 tunes the Ca2+ -sensitivity and open state of its channel, it has been proposed as a regulator of K+ efflux. Since K+ channels have been implicated in cell stiffness changes, we proposed KCNMB1 contributes to cell stiffness by promoting K+ efflux and increasing membrane tension. MATERIALS or METHODS: We utilized both in vivo and in vitro models to explore the effects of K+ channel agonism on mouse breast cancer cells. Cancer and cytotoxic lymphocyte cocultures and mouse models were both used. RESULTS: In our effort to exploit this therapeutic vulnerability, we used a BK channel agonist, BMS-204352 to increase target cell stiffness and reduce tumor growth. CONCLUSIONS: Overall, our results suggest K+ channel agonism slows tumor development, but the mechanism underlying this phenomenon remains incompletely understood.

Poster 5

Vipin Rawat

Therapeutic enzyme depletion of L-serine for the treatment of serine auxotrophic tumors

Therapeutic enzyme depletion of L-serine for the treatment of serine auxotrophic tumors Vipin Rawat1, Huiping Zhao1, Ladan Mashouri2, Prarthana Prasanth1, Kelly Conger1, Sarita Bhetawal2, Alessandra Araujo2, Everett Stone2, Jonathan Coloff1 1Physiology and Biophysics, University of Illinois College of Med. at Chicago, Chicago, IL,2University of Texas at Austin, Austin, TX ABSTRACT: Serine, a non-essential amino acid is one of the most important nutrients in cancer cells. Serine is utilized not only as one of twenty amino acids required for protein synthesis, but also for the synthesis of nucleotides, lipids, and antioxidants. As a non-essential amino acid, serine can either be synthesized de novo or taken up from outside of the cell. Initial efforts of targeting serine metabolism were aimed at inhibiting serine synthesis using serine synthesis enzyme specific inhibitor. However, numerous recent reports (including our own) have shown that in most cases inhibition of serine biosynthesis is not an effective treatment when extracellular serine is present, as exogenous serine is also capable of supporting cancer cell proliferation. The importance of exogenous serine in tumors has motivated investigation of serine starvation as another potential method of targeting serine metabolism in cancer. While most cancer cells appear to be able to synthesize serine de novo, serine auxotrophy can be induced by certain genetic alterations or by epigenetic silencing of serine synthesis genes, as we have recently discovered in luminal breast cancer (ER+). At present, due to the pathway redundancy only way to reduce serine availability for tumors in vivo is through dietary serine starvation. well-tolerated by mice, consumption of a 100% synthetic protein-free diet may prove challenging for humans due to known non-adherence issues in most cancer dietary restriction trials. Additionally, dietary serine starvation only achieves a 50% reduction in circulating serine levels, limiting its use in cancer patients. Due to the inherent challenges associated with dietary serine starvation, we engineered human serine dehydratase. The enzyme serine dehydratase facilitates the pyridoxal phosphate-dependent, irreversible deamination of serine into pyruvate and ammonia, therefore reducing serine levels in culture and mice. BACKGROUND, PURPOSE, or OBJECTIVES: At present, only way to reduce serine availability for tumors in vivo is through dietary serine starvation. well-tolerated by mice, Additionally, dietary serine starvation only achieves a 50% reduction in circulating serine levels, limiting its use in cancer patients. Development of a therapeutic serine degrading enzyme will remove the requirement of dietary change and effective reduction in serine levels. HYPOTHESIS/AIMS: Although dietary serine deprivation could be potentially beneficial for cancer patients, several inherent limitation still exist. To overcome these challenged , we have developed a therapeutic enzyme capable of effectively reducing circulating serine levels. MATERIALS or METHODS: In this work, we make use of serine auxotrophic cells line and tumors to test the efficacy of our enzyme in culture and in mice. RESULTS: Our engineered version (eSDH) has increased activity against serine when compared to threonine. Enzyme engineering also increased it stability for extended circulatory persistence. The injection of the eSDH enzyme can achieve a long-term, near-complete (>90%) reduction in circulating serine levels.

Poster 7

Didi Zha

Modeling the malignant transformation of fallopian tube epithelium driven by extracellular vesicles cargos in an organ-on-chip microphysiological system

ABSTRACT: BACKGROUND, PURPOSE, or OBJECTIVES: Epithelial ovarian cancer (EOC) remains the leading cause of death from gynecological malignancy and the fifth leading cause of all cancer-related deaths among American women. The most common and lethal subtype, high-grade serous ovarian cancer (HGSOC) tends to originate from the fallopian tube. We are interested in understanding if ovarian cancer initiation in the fallopian tube epithelium (FTE) is mediated by extracellular vesicles (EVs), which are nanosized biovesicles secreted by all living cells. EVs serve as vehicles for nucleic acids, proteins, and lipids. Cancer cells commonly secrete more EVs than healthy cells and shift their EV cargos to promote tumorigenesis. Tumor-derived EVs represents a general mechanism that cancer cells alter the characteristics of neighboring healthy cells and tumor microenvironment to favor tumor progression. However, the ability of extracellular vesicles from tumor to transform healthy tissue into cancerous lesion is less understood. HYPOTHESIS/ AIMS: Our hypothesis is that extracellular vesicles cargos secreted by tumor cells contain factors promoting tumorigenesis in the FTE. Our goal is to interrogate the ability of EVs in the initiation of ovarian cancer in the FTE at the early stage, for which there is no adequate in vitro model. MATERIALS or METHODS: We create a microfluidic model using a 3D dynamic culture system termed PREDICT-MOS to enable the direct interaction of FTE with EVs and the long-term culture of the fallopian tube explant. We are interested to 1) test the role of EVs to drive preneoplastic transformation of FTE progenitor cells and 2) to test whether tumor derived-EVs shift cargos from FTE. The hFTE cultured on PREDICT-MOS is exposed to cancer cell-derived EVs and embedded for transcriptomic profiling utilizing GeoMx Digital Spatial Profiler to identify differentially expressed genes in both ciliated and secretory cells within the epithelium. RESULTS: Our preliminary data demonstrates human fallopian tube epithelium exposed to EVs from ovarian cancer cells upregulates the expression of RNA transcripts involved in pro-inflammatory response and pro-angiogenesis, which are important in immune regulation and early tumor formation. We have validated the upregulated transcripts in immortalized fallopian tube cells using real-time qPCR and the increased expression at protein level in human fallopian tube tissue using immunohistochemistry. Additionally, the longer exposure to EVs from ovarian cancer cells allows the fallopian tube tissue to shift the molecular content secreted via extracellular vesicles. After 14-day treatment with EVs from cancer cells, we have found fallopian tube tissue significantly increased the secretion of twenty-eight EV proteins. These proteins promote glutamine uptake, angiogenesis, wound healing and negatively regulate cell-to-cell adhesion, which are important for early tumorigenesis. CONCLUSIONS: Ovarian cancer cell-derived extracellular vesicles alter the gene expression and the molecular cargos of healthy fallopian tube. Further analysis and validation of fallopian tube EV proteomics and transcriptomic profile are needed to establish their association with high-grade serous ovarian cancer. This project is supported by Ovarian Cancer Research Fund Alliance Program Grant.

Poster 8

Nivida Shete

Impact of Estrogen Receptor Targeting Drugs on a Novel Epithelial-Mesenchymal Transition (EMT) Pathway in Endocrine Resistant Breast Cancer: Implications for Metastasis

Background: Approximately 10% of breast cancer patients are metastatic at the time of diagnosis with a 27% survival rate. Estradiol (E2) treatment was discontinued due to its bad safety profile and the introduction of the safer drug, tamoxifen. Currently, endocrine therapy, including Selective Estrogen Receptor Modulator (tamoxifen), aromatase inhibitors (letrozole, anastrozole, and exemestane), Selective Estrogen Receptor Degraders (fulvestrant and elacestrant), is the standard of care for patients with estrogen receptor-positive (ER+) breast cancer. The major treatment challenge is endocrine therapy resistance and metastasis. Metastasis is caused by the migration of cancer cells from the primary tumor to other tissues and organs. EMT is the process in which cells lose epithelial properties and acquire motility and mesenchymal phenotype, which leads to migration and invasion. EMT is characterized by the destabilization of adherens junctions by loss of E-cadherin at the plasma membrane. A novel signaling pathway dominant in tamoxifen-resistant (TR) breast cancer cells involves transcriptional repression of p120catenin by upstream regulators PKCa and FOXC2, resulting in loss of E-cadherin at the membrane (Pham, 2017). EMT in these models was confirmed by decreased expression of epithelial markers and increased migration and invasion. The impact of various ER-targeting drugs including E2, tamoxifen, fulvestrant, and a novel Selective Human Estrogen Receptor Partial Agonist, TTC-352, on the novel PKCa-FOXC2-p120catenin EMT pathway has not been studied yet. Phase I clinical trial of TTC-352 has shown a better side effects profile and an average of 57 days of progression-free survival (PFS), demonstrating efficacy without reaching the maximum tolerated dose (Dudek, 2020). This study will help identify effective therapeutic approaches to treat aggressive ER+ breast cancer. Strategizing treatment with endocrine drugs could potentially reduce the recurrence of tumors and may reveal a set of biomarkers predictive of treatment response. Hypothesis: ER-targeting drugs modulate the novel EMT pathway in TR breast cancer cells. Materials and Methods: Cell lines used in this study include PKCa overexpressing, MCF-7/PKCa and MCF-7:5C representing TR, migratory ER+ breast cancer. Migration assay in response to ER ligands was performed using Falcon Permeable inserts where the cells were seeded after the treatment. Migrated cells in response to chemoattractant in the well were stained and counted after 24 hours. Luciferase reporter assays were performed to evaluate the effect of ER ligands on p120catenin transcription. The cells were transiently co-transfected with the p120catenin promoter plasmid and renilla vector for normalization. FOXC2 binding on the p120-catenin promoter causes repression of its transcription. Luciferase luminescence was measured on a plate reader. Results: E2 and TTC-352 treatment increased the p120catenin transcription and decreased the migration of MCF-7/PKCa and MCF-7:5C breast cancer cells compared to vehicle and other ER ligands - tamoxifen and fulvestrant. Next, using chromatin immunoprecipitation, I will confirm reduced FOXC2 binding on the p120catenin after E2 and TTC-352 treatment. Conclusion: Patients with endocrine-resistant ER+ breast cancer may benefit from TTC-352 treatment by inhibiting the activation of a novel EMT pathway. Strategizing less toxic treatment options would potentially prolong patient survival with a better quality of life.

Poster 9

Kihak Lee

Expression of MRTFA facilitates an immunosuppressive tumor microenvironment via upregulation of VSIR

ABSTRACT: BACKGROUND, PURPOSE, or OBJECTIVES: Breast cancer is the second leading cancer in women, accounting for 31% of women's cancers. Despite similar incidence rates of breast cancer, African American women have been shown to have a 4 to 5 times higher risk of death compared to their White American counterparts, highlighting a significant disparity in survival outcomes. Metastasis of breast cancer cells to other organs is the primary cause of death of breast cancer patients and cancer metastasis is regulated by transcription factors. For example, myocardin-related transcription factors A and B (MRTFA/B) are strong regulators of actin polymerization. Accordingly, they are involved in cancer cell motility, migration, and metastasis. However, paradoxically, MRTFA/B expression also renders cancer cells vulnerable to cytotoxic lymphocytes (CTLs). In addition, MRTFA/B is upregulated in cancer associated fibroblasts, which contribute to immune regulation, and extracellular matrix remodeling of the tumor, encouraging tumorigenesis. This dual function of MRTFA/B and their expression in the cells of the tumor microenvironment (TME) underscores the importance of examining MRTFA/B's expression patterns and functions in both tumor cells and the cells of the TME. HYPOTHESIS/AIMS: MRTFA/B expression in breast cancer TME leads to immune dysfunction and metastasis. MATERIALS or METHODS: To explore the clinical, demographic, tumor-intrinsic and tumor microenvironmental patterns of MRTFA/B's expression and activation, we utilized multiplex imaging methods on multiple racially diverse tissue microarrays (TMA) and bioinformatics analyses of The Cancer Genome Atlas (TCGA), Molecular Taxonomy of Breast Cancer International Consortium (METABRIC), and available single cell RNA sequencing datasets. RESULTS: We investigated MRTFA's expression pattern in TME by using single cell sequencing database and found more abundant MRTFA expression in the dendritic cells in TME among diverse cell subtypes in TME. MRTFA expression in bulk tumors also correlated positively with dendritic cell (DC) infiltration in the TCGA dataset. Moreover, our multiplex-imaging of TMA samples revealed that MRTFA is prominently expressed in perivascular HLA-DRA+CD45+ cells, indicative of antigen presenting cells. Notably, public single cell RNA sequencing data revealed that breast cancer DCs strongly express V-set immunoregulatory receptor (VSIR), which is one of immune check proteins, implying the immunosuppressive TME. We also found a higher MRTFA expression in stromal cells of lymph node metastasis regions using our TMA samples and The Cancer Genome Atlas (TCGA) dataset. In addition, we found an increased T-cell infiltration, and T-cell dysfunction in African American patients with elevated VSIR gene expression in TCGA dataset. CONCLUSIONS: MRTFA expression in DCs in breast cancer could contribute to breast cancer disparities between African American and White American women by comprising immunosuppressive TME through upregulation of VSIR expression. We will test this hypothesis by functional work in human and mouse dendritic cells.

Poster 10

Mete Emir Ozgurses

Generation of a human physiological lipid media to improve in vitro studies of lipid metabolism.

BACKGROUND, PURPOSE, or OBJECTIVES: Lipids are crucial for cellular function as they are the main structural components of cellular membranes and play critical roles in metabolism, signaling, and other core processes. Many studies of lipid metabolism in cancer rely on the results of in vitro tissue culture experiments. Typically, the only source of lipids in traditional media (TM) is bovine serum, typically supplemented at 5-15%. Thus, cells cultured in these conditions are subject to an ~85-95% reduction in lipid exposure relative to human serum. Recently, several groups have generated culture media with physiological levels of polar metabolites, which can correct some tissue culture-related artifacts. However, these new-generation media still use traditional serum and still represent severe lipid starvation conditions. In lipid-deprived environments, cells respond by activating the sterol regulatory element binding protein (SREBP) pathway, a transcriptional regulator of global lipid metabolic gene expression. Our SREBP transcription factor signature scores comparing human tumor samples to established cancer cell lines growing in TM showed dramatic activation of SREBP in cell lines relative to tumors, supporting traditional tissue culture being a highly lipid-starved environment. HYPOTHESIS/AIMS: We hypothesize that the lipid-starved conditions found in traditional ~10% FBS culture media impact cancer cell behavior in vitro and that the development of a more physiological human serum lipid medium will maintain more accurate cell metabolic characteristics. MATERIALS or METHODS: We generated Human Serum Lipid Media (HSLM) according to human serum lipoprotein levels and compared it to human samples and TM with lipidomic analysis. Cells were then grown in HSLM and TM to obtain growth curves. We have further studied the effects of HSLM on cancer cell survival using colony formation assays and PI/ Hoesct staining. Lastly, to assess available lipoproteins in the tumor microenvironment we isolated both plasma and Tumor Interstitial Fluid (TIF) from mouse breast tumor models and analyzed lipoprotein expression by western blot. RESULTS: Our lipidomics analysis showed that the HSLM is highly similar to human serum samples relative to TM. However, we found that HSLM induced cell death in multiple breast cancer cell lines. Our analysis of lipoprotein levels in TIF suggests that lipid exposure is significantly reduced in the tumor microenvironment relative to serum, suggesting that tumor cells may not normally be exposed to such high levels of lipids. A likely exception to this is during metastasis where cancer cells will be exposed to serum levels of lipids while in circulation. As such, we wondered whether cells will be less sensitive to HSLM in suspension, as this is their natural state while in circulation. Indeed, colony formation assays showed that cancer cell lines are less sensitive to HSLM in suspension compared to the attached conditions. CONCLUSIONS: Our work suggests that HSLM better represents human lipid levels than TM. However, cells grown in attached conditions die when exposed to HSLM, while cells in suspension are more resistant. These results suggest that cells in their normal tumor environment are likely not exposed to full serum levels of lipoproteins and that cells in suspension take on a

Poster 11

Sworaj Sapkota

The synergistic effect of cadmium and epithelial-fibroblast crosstalk in prostate cancer progression

Background Cadmium is a ubiquitous cancer-causing agent found in air, water, soil, and food [1]. Studies have shown that dietary as well as occupational exposure to cadmium leads to its accumulation in the body [2]. The role of cadmium (Cd) in the initiation of prostate cancer (PCa) is well established however research in this domain has majorly been restricted to the prostatic epithelium. This approach fails to consider the effects of cadmium on other prostatic cell types, primarily the fibroblast cells which surrounds the epithelium. Thus, not much data is available on the role of cadmium in PCa with regards to the prostatic epithelial and fibroblast cells in combination. Hypothesis Although chronic exposure to cadmium is known to have some effect on fibroblast cells, we hypothesize that the cadmium-initiated (CI) transition of prostatic fibroblast cells to a cancer associated fibroblast (CAF) phenotype relies on the synergy between the pre-malignant induction of epithelial cells by cadmium and subsequent epithelial-fibroblast crosstalk (EFC) making CI PCa a two-stage process. Methods In this work, we focus on studying the effects of direct and indirect cellular interactions between prostatic epithelial and fibroblast cell lines chronically exposed to cadmium (WPMY-1 and BPH-1 renamed WPMY-Cd and BPH-Cd following cadmium exposure) along with PC-3, a metastatic prostatic epithelial cell line. In vitro 3D models were used to assess the sprouting ability of WPMY-Cd in the presence of BPH-Cd or BPH-Cd secretions while 2D in vitro models were used for cell-cycle and cell proliferation analysis by flow cytometry, and protein expression analysis by immunoblotting and qPCR in similar conditions. Results We observed higher rates of WPMY-cd cell proliferation and a shift to G2/M phase in the presence of BPH-Cd and PC3 cells compared to WPMY-Cd cells alone in flow cytometry studies. In addition, 3D tumor spheroid analysis showed increased invasive capabilities of WPMY-Cd spheroids in the presence of BPH-Cd cell secretions. Our data also showed an up-regulation in the expression of Fibroblast Activation Protein (FAP), _-Smooth Muscle Actin (_-SMA), PDGFR-_, SOX2 and B-catenin proteins in WPMY-Cd cells. On the other hand, co-culture studies showed increased expression of SOX2 in BPH-Cd cells, and increased expression of both SOX2 and ALDHA-1 in PC3 cells. Taken together, our finding indicates not only the endowment of CAF-like characteristics in fibroblast cell lines but also an increase in cancer stem-cell (CSC) like characteristics in epithelial cell lines. More importantly, our data points to the potential activation of a positive feedback loop between epithelial and fibroblast cells chronically exposed to cadmium and a subsequent increase in prostate cancer aggression. Conclusions Further protein array analysis is in progress to delineate the signaling mechanisms orchestrated by the synergy between cadmium and EFC in promoting PCa progression. Therefore, strategies to disrupt this feedback loop could prove to be highly effective therapeutic interventions to manage CI PCa

Poster 12

Monica Haughan

Adrenergic Signaling in Adipocytes Drives Ovarian Cancer Cell Invasion

BACKGROUND: Despite low prevalence, ovarian cancer (OC) is the 5th leading cause of cancer death in women. High grade serous OC (HGSOC), the most common and lethal OC subtype, is typically diagnosed after it has spread. HGSOC habitually spreads to the omentum, a fatty tissue covering the abdominal organs. Consequently, we seek to uncover metabolites involved in omental spread and their mechanisms to identify potential therapeutic targets. We found epinephrine, aka adrenaline, is increased when HGSOC cells are cultured with omentum. Known epinephrine signaling via adrenergic receptors causes lipid release and lipids released from fatty tissues are known to feed cancer cells. As there is already a well-tolerated, FDA approved, medication which blocks epinephrine signaling-the beta blocker, propranolol-, investigating the role of epinephrine signaling in HGSOC spread has translational promise. HYPOTHESIS: We hypothesize the observed increase in epinephrine is produced omental adipocytes causing increased omental lipid release in turn increasing HGSOC spread. METHODS: To understand the communication between HGSOC and the omentum we leverage a cutting-edge technology known as imaging mass spectrometry (IMS) which retains spatial data while identifying metabolites in a sample. We apply IMS to a co-culture system where the HGSOC cell model, MOE PTENshRNA, is cultured with mouse omentum. IMS then allows us to identify the metabolites produced in this condition compared to nontumorigenic and cell of origin controls and identify the source of the metabolite. We use a 3D spheroid forming, human adipocyte cell model to simulate omental adipocytes. Murine omentum and adipocyte spheroids were treated with epinephrine (10QM), propranolol (1QM), or a vehicle control. The resulting conditioned media (CM) was collected. These CM were then used as a treatment on three HGSOC cell lines (MOE PTENshRNA, OVCAR4 and OVCAR8) to investigate the impact on HGSOC invasion via Boyden chamber assay and HGSOC viability via SRB assay. RESULTS: IMS revealed epinephrine as one signal unique to the MOE PTENshRNA + omentum condition. Invasion of MOE PTENshRNA, OVCAR4 and OVCAR8 was increased with untreated CM compared to the serum free control. Further, invasion with propranolol treated CM from both omentum and adipocyte spheroids was decreased. However, only epinephrine treated omental CM increased MOE PTENshRNA invasion. All CMs increased HGSOC cell viability in starved conditions, however there was no significant difference between the treated CMs. CONCLUSIONS: Overall, we are working to understand the impact of the epinephrine identified in our IMS co-culture system on HGSOC. We have shown that blocking adrenergic signaling in omentum and adipocyte spheroids with propranolol decreases the invasion stimulated by their CM, while epinephrine treatment of omentum increased the invasion stimulated. However, as the treatments did not impact viability this suggests specific secreted factors released by adipocytes induce different HGSOC cell behavior important in disease progression. Our findings suggest propranolol warrants further investigation as an agent to potentially mitigate HGSOC spread. Next steps include identifying the secreted factor responsible for these changes.

Poster 13

Manead Khin

Wheldone, a fungal secondary metabolite, leads to mitotic catastrophe

Ovarian cancer, the fifth leading cause of cancer-related death in women, is the most aggressive gynecological malignancy in the world. Among the various subtypes of ovarian cancer, high-grade serous ovarian cancer (HGSOC) is the most common and lethal. This lethality is primarily due to lack of early detection and frequent chemoresistance. Hence, it is crucial that novel therapies are developed. Natural products contribute to over 70% of all anti-cancer drugs developed and are an important source for new drug leads. Wheldone, a compound isolated from fungi, shows promise in leading to ovarian cancer cell death. Wheldone triggered apoptosis, programmed cell death, in HGSOC cells. Moreover, wheldone led to DNA damage in the cancer cells. When quantitative proteomics was performed, wheldone significantly regulated microtubule regulation, mitotic spindle assembly, and chromosomal condensation during mitosis. Cell cycle analysis showed that G2/M arrest was induced by wheldone, supporting a change in mitosis. Therefore, our current hypothesis is that wheldone triggers mitotic catastrophe, ultimately leading to DNA damage and resulting in apoptosis. Wheldone belongs to a novel chemical compound class for which the mechanism remains unknown, suggesting that wheldone may have a unique target. Therefore, future studies will prioritize elucidating wheldone's molecular target and confirming how that target is consistent with the comprehensive differential proteomics studies.

Poster 14

Purab Pal

Ceramides induce a lethal level of endoplasmic reticulum stress to preferentially kill endocrine therapy-resistant breast cancer cells.

BACKGROUND, PURPOSE, or OBJECTIVES: Endocrine Therapy (ET)-resistant breast cancer remains a clinical problem, with approximately 40% of patients experiencing disease relapse. In a recent study, we demonstrated that preclinical models of ET-resistant breast cancer have an altered sphingolipid profile, including low levels of endogenous ceramides, and are more sensitive to ceramide-induced cell death compared to ET-sensitive models. The current study is designed to identify mechanisms by which ceramides preferentially induce cell death in ET-resistant breast cancer models in order to identify novel therapeutic strategies to improve ET-resistant breast cancer patient outcomes. HYPOTHESIS/AIMS: As a bioactive lipid, ceramides can interact with membrane lipids or cellular proteins to exert its biological actions. In our current study, we focused on ceramide-protein interactions and investigated if ceramide interacts with proteins that are specifically important for ET-resistant cell survival. MATERIALS or METHODS: We used a photoactivable-and-clickable ceramide probe and quantitative proteomics to identify ceramide-interacting proteins (CIPs) that are differentially expressed in ET-resistant breast cancer cells. Ceramide-induced effects on the transcriptome were assessed by performing total RNA-seq on a panel of ET-sensitive and -resistant cell lines with or without ceramides. RESULTS: We found that 946 out of 1220 CIPs are enriched in ET-resistant compared to ET-sensitive cells, and that the top enriched CIPs include known ET-resistant survival proteins, such as ERBB2, PKC_, and IKKß. While most of the CIP-ceramide interactions and their downstream consequences have yet to be characterized in the context of ET-resistance, we have observed that a group of clinically relevant CIPs are located in the endoplasmic reticulum (EnR) membrane. Higher expression of these CIPs is associated with more Luminal B disease as well as worse relapse-free patient survival. While most of these CIPs have yet to be implicated in ET resistance, a subset of these CIPs is known to be involved in attenuating endoplasmic reticulum stress (EnRS). To assess whether ceramides affect EnRS, we performed an RNA-seq study and found that the top regulated genes and pathways differentially regulated by ceramide in ET-resistant vs. ET-sensitive cells are associated with EnRS. To test whether ceramide-induced EnRS was mediated by CIPs, we performed siRNA-mediated knockdown of one of the top CIPs in the ER-membrane, TRAM1. We found that TRAM1 knockdown phenocopies ceramide treatment by significantly increasing cell death and a higher level of EnRS specifically in ET-resistant but not in ET-sensitive cells. These findings suggest that ceramide interaction with ER-membrane proteins, such as TRAM1, may disrupt their function to induce a lethal level of EnRS in ET-resistant cells. CONCLUSIONS: Together, we find that ceramide induces preferential cell death in ET-resistant cells through interactions with a novel array of proteins leading to the inhibition of key survival proteins and activation of lethal levels of EnRS. Understanding how ceramides interact with and affect these proteins could lead to the development of novel strategies to selectively treat ET-resistant breast cancers and improve patient outcomes.

Poster 15

Philippa Burns

Serine Starvation Inhibits SRSF Protein Expression and Modulates RNA Splicing

BACKGROUND: Breast cancer is the most common cancer in women worldwide, and luminal breast cancer accounts for ~50% of breast cancer deaths. Our lab has identified that luminal breast cancer cells are dependent on the amino acid serine from the diet as they are unable to make it. Serine is utilized for making proteins, nucleotides, and lipids, and so is critically important for cancer cell growth. This makes targeting serine metabolism, for example by serine starvation, a promising potential therapy for luminal breast cancer, and various other serine-dependent tumors, to limit tumor growth. HYPOTHESIS/AIMS: While the metabolic effects (on nucleotides and lipids) of serine starvation have been extensively investigated, our goal is to better understand how serine starvation affects proteins. This will help us better understand what serine is important for, as well as the effects of manipulating serine metabolism on cells. We hypothesized that synthesis of serine-rich proteins would be particularly affected by serine starvation. MATERIALS/METHODS: MCF7 luminal breast cancer cells were cultured in the presence or absence of serine and glycine (S/G). To study effects of serine starvation on overall protein synthesis, we performed puromycin incorporation and polysome fractionation assays. The amino acid deprivation response was studied by immunoblotting for ATF4. Premature termination and ribosome stalling reporters were used to study translation efficiency at serine codons. We took the approach of proteomics analysis (validated by western blot) to identify proteins that may be particularly affected by serine starvation, and performed pathway analysis on these proteins. As we found that serine starvation downregulates mRNA splicing pathways, we performed analysis on RNA-sequencing data to study changes in alternative splicing events. CRISPR-Cas9 was used to knockout serine tRNA synthetase (SARS1) and serine/arginine-rich splicing factor 6 (SRSF6) to study whether splicing changes upon serine starvation are through the protein synthesis function of serine and subsequent decrease in serine-rich proteins (e.g. SRSF6). Expression of serine synthesis enzyme PSAT1 was manipulated to study effects of serine dependency. We studied the DNA damage response by immunoblotting for p-H2AX, and measured cell death by propidium iodide and Hoechst staining. RESULTS: We found that serine starvation inhibits overall protein synthesis, while inducing ATF4, and causes ribosome stalling and fall-off at serine TCC codons. Serine starvation particularly depletes serine-rich proteins, including a family of serine/arginine-rich splicing factors (SRSFs) which are important in mRNA splicing. SARS1 knockout reduces protein synthesis and expression of SRSF6, like serine starvation. Serine starvation downregulates mRNA splicing pathways in MCF7 cells, and dramatically alters mRNA splicing in multiple breast cancer cell lines. Knockout of SRSF6 induces changes in mRNA splicing which overlap with serine starvation, in genes associated with DNA repair. SRSF6 depletion reduces DNA damage response and increases cell death under serine starvation and with various chemotherapy drugs. CONCLUSIONS: Serine starvation induces changes in pre-mRNA splicing due to reduced translation of serine-rich members of the SRSF family (such as SRSF6), which ultimately impacts DNA damage and cell death. We believe that SRSF6 depletion upon serine starvation may sensitize to chemotherapy.

Poster 16

Mohamed Haloul

The role of ISG15 in breast cancer metastasis

Abstract: Background: Breast cancer metastasis presents a formidable challenge for prognosis and treatment. While there has been progress in understanding the molecular aspects, developing effective therapies remains arduous. Alterations in the biophysical properties of cancer cells can influence immune responses. Our recent research suggests that increased cellular rigidity, resulting from actin polymerization and stress fiber formation, enhances immunosurveillance. Interferon-stimulated gene 15 (ISG15), similar to ubiquitin, exists in intracellular, conjugated (ISGylation), and extracellular forms. ISG15 is overexpressed in breast cancer tissues and correlates with poor prognosis, advanced tumor grade, and invasiveness. However, the specific mechanism by which ISG15 promotes breast cancer metastasis and its role in modulating the tumor microenvironment are not fully understood. OBJECTIVES: This study aimed to investigate the role of ISG15 in regulating the biophysical properties of breast cancer cells and its impact on metastasis and immune evasion. HYPOTHESIS/AIMS: We hypothesized that ISG15 induces changes in cell rigidity, facilitating immune evasion and metastasis in breast cancer. MATERIALS AND METHODS: We generated ISG15 knockdown and knockout cell lines, along with ISG15-WT and ISG15 mutant overexpressing cells. We modulated ISGylation by manipulating the expression of key enzymes UBA7 and UBE2L6. Cell stiffness was measured using Atomic Force Microscopy (AFM), and F-actin stress fibers were visualized using phalloidin staining. In a metastasis experiment, mice were injected with control or ISG15 knockdown cells, and metastasis was quantified using LagoX imaging. We used Western blot to assess the activity of actin cytoskeleton regulators, including ERM, MLC2, and cofilin. We will investigate the immunosurveillance using the killing assays and utilizing the immunocompromised and immunocompetent mice. This study is unique in that it is the first to use ISG15 knockout (ISG15-/-) mice in the context of breast cancer. RESULTS: In our study, ISG15-/- cells demonstrated a significantly higher number of F-actin stress fibers, a greater F-actin/G-actin ratio, and increased cell size compared to CRISPR-control cells, indicating heightened cell stiffness. This increased stiffness was confirmed using Atomic Force Microscopy (AFM). Additionally, ISG15 knockdown induced phosphorylation of ERM, MLC2, and cofilin, suggesting a role for ISG15 in regulating cell stiffness through these actin cytoskeleton regulators. LagoX imaging revealed that ISG15 knockdown decreased metastasis compared to the control, indicating a pro-metastatic role for ISG15. Furthermore, ISG15-/- mice injected with E0771 cells showed reduced tumor volume and metastasis compared to wild-type mice, highlighting the importance of ISG15 in promoting breast cancer metastasis. CONCLUSIONS: These results collectively suggest that ISG15 plays a crucial role in regulating the biophysical characteristics of breast cancer cells, influencing metastasis and potentially immune evasion. Targeting ISG15-mediated changes in cell properties could be a promising therapeutic approach for combating breast cancer metastasis.

Poster 17

Jeff Kim

From Fibrosis to Cancer: Uncovering the Pathogenesis of Hepatocellular Carcinoma in Metabolic Associated Steatohepatitis

BACKGROUND/PURPOSE/OBJECTIVES: Between 1% and 3% of patients with end-stage Metabolic Associated Steatohepatitis (MASH) progress to Hepatocellular Carcinoma (HCC), a transition that is not well understood. The lack of comprehensive insight into this transition hampers our ability to predict, prevent, and effectively treat the progression, leading to potentially dire consequences for patient outcomes. HYPOTHESIS/AIMS: This study aims to elucidate the cellular and molecular mechanisms that underlie the transition from end-stage MASH to HCC, paving the way for the development of targeted interventions that can mitigate this progression and improve patient prognoses. MATERIALS/METHODS: We analyzed two patient bulk RNA-seq datasets (n=359), applying MuSiC2 for cell type deconvolution and conducting comprehensive bulk RNA-seq analysis to identify cellular and genetic alterations. This approach enabled the identification of distinctive cell type dynamics and the expression patterns of cancer-related marker genes across the disease spectrum, from healthy liver to Stage 4 MASH. RESULTS: Our analysis revealed significant cell type dynamics correlating with the disease progression. Specifically, we noted a trend of escalating proportions of certain cell types from healthy liver to Stage 4 MASH. This trend was particularly pronounced in immune cells, marked by progressively increasing proportions of TREM2+ tumor-associated macrophages, plasma B cells, and T cells. In NASH Stage 4, there was an augmented presence of specialized endothelial cells, such as PDPN+ lymphatic and CADM+ vascular variants, and heightened expressions of PBK+ cells responsible for cell cycle regulation. Furthermore, our analysis of bulk RNA-seq data indicated the upregulation of cancer marker genes typically associated with multiple neoplasms, as well as those specifically identified in HCC. These cancer-related genes exhibited significantly high expression in Stage 4 of MASH, offering clear insights into potential mechanisms driving the progression from end-stage fibrosis in MASH to HCC. CONCLUSIONS: This study provides a deeper understanding of the cellular and molecular landscape during the transition from MASH to HCC. By identifying distinctive cell type dynamics and the expression of cancer-associated genes in the end-stage of MASH, we illuminate previously unidentified pathways that contribute to the development of HCC. These findings open new avenues for creating targeted therapeutic interventions and preventive strategies, potentially transforming the management and outcomes of MASH patients facing the risk of HCC.

Poster 19

Myna Adhikari

Induction of Mutations by Human Topoisomerase II

Background: Topoisomerase II (Top2) regulates DNA topology by introducing transient breaks in DNA. While the topoisomerases mechanism is a “safe” way to cut DNA, failure of this mechanism by small molecules or random processes can lead to genome instability. Mammalian cells express two Top2 enzymes: Top2_ and Top2_, and it has been suggested that Top2_ can generate long-lasting DNA breaks in some contexts. A new approach to studying how topoisomerases affect genome integrity is the isolation of alleles of Top2 that generate elevated levels of DNA cleavage. Interestingly, this type of mutation has been identified in both Top2_ and Top2_ in human cancer cells. The goal of this work is to explore the genome instability induced by Top2_ mutants. Hypothesis: Expression of Top2_ hyper cleavage alleles will generate elevated levels of mutations, and the mutations may include de novo duplications as was seen with other hyper cleavage Top2 mutants. Human Top2_ expression may also lead to mutation types that have not been detected with other topoisomerases. Methods: The human Top2_ gene was constitutively expressed in yeast cells with either wild type or a temperature sensitive allele (top2-4) of yeast TOP2. The hyper-cleavage alleles examined in this work include Top2_-K600T, Top2_-R757W and Top2_-V111I. Expression of was examined by Western analysis. Mutation rates were determined by quantitation of colonies resistant to canavanine. Canavanine resistance arises primarily by mutations in CAN1, which encodes arginine permease. The nature of mutations induced . To determine the nature of mutations, a LYS2 frameshift reversion assay was used, that selects for frameshift mutations within a small segment of the yeast LYS2 gene. The LYS2 segment was then sequenced by Sanger sequencing or using a novel multiplex approach. Results: Expression of Top2_ was examined in TOP2+ and top2-4 of yeast strains. In TOP2+ strains, 20/20 isolates expressed wild type Top2_, 12/20 isolates expressed Top2_-K600T, 13/20 expressed Top2_-R757W and 12/20 expressed Top2_-V111I. This result highlights the necessity of using the temperature sensitive top2-4 allele to ensure that the Top2_ allele is being expressed. Preliminary experiments for measuring the frequency of CAN1 mutations in TOP2+ and top2-4 cells are underway. In TOP2+ yeast cells, Top2_-K600T expression led to a 5.8 fold increase in canavanine resistant mutations, while expression of Top2_-K600T in top2-4 cells led to a 4.6 fold increase in canavanine resistant mutations. Detailed mutation rates determination for Top2_-K600T, Top2_-R757W and Top2_-V111I is currently in progress. We have adapted a multiplex sequencing assay from a commercial vender to sequence 5-20 DNA samples at the same time and have shown accurate detection of nucleotide insertions in 7 different samples. Preliminary assessment of mutation spectra of Top2_ alleles will be presented. Implications: Our preliminary results demonstrate that hyper cleavage alleles of Top2_ induce genome instability that includes induction of insertions and deletions. Since Top2_ is expressed in all human cells, we suggest that Top2_ may be a relevant factor in genome instability.

Poster 20

Jorge Heneche

Cole Relaxation Frequency as an Earlier Detection Biomarker of Pancreatic Neoplasia prior to development of Pancreatic Cancer

BACKGROUND: For most solid tumors, early detection provides an improved therapeutic window leading to increased survival for cancers like breast, prostate, and colon. Unfortunately, there are few if any modalities that provide such an opportunity for pancreatic cancer (PC), which continues to increase in incidence and mortality. NovaScan has engineered a device that measures spectral bioimpedance to extract the Cole Relaxation Frequency (CRF). This parameter has been tested in breast and lung cancers as a biomarker for cancer detection, demonstrating increased CRF values 2 orders of magnitude higher for malignant compared to benign tissue. In our preliminary study, we used ex vivo mouse PC to confirm a similar increase in pancreatic CRF value between wild type and LSL-KrasG12D; LSL-p53R172H ; Pdx1-Cre (KPC) mice. HYPOTHESIS: The Cole Relaxation Frequency (CRF) will serve as an effective biomarker for early pancreatic cancer detection, differentiate between pancreatic cancer and acute pancreatitis with high accuracy, and correlates with pancreatic fibrosis, aiming to elevate diagnostic accuracy and patient outcomes in pancreatic cancer. METHODS: The biomarker was evaluated in a double blind study on ex vivo pancreata of mice: 15 KPC, 2 LSL-KrasG12D/Pdx1-Cre (KC), and 9 wild type controls (Study 1). To determine if this bioimpedance biomarker can distinguish between PC and acute pancreatitis (AP), we challenged it with cerulein-induced AP and 6 saline-injected control mice (Study 2). In both mouse studies, fibrosis was evaluated for a correlation with the CRF biomarker. To extend this approach in humans, a double-blinded study of 30 patients (11 males, 19 females) undergoing EUS procedures with FNB sampling for suspected pancreatic lesions were assessed by nsCanary and validated against pathology outcomes (Study 3). RESULTS: The results from Study 1 we confirmed via histopathology 12 KPC pancreases as cancerous, 9 controls as noncancerous, and 5 (3 KPC and 2 KC) presenting with PanINs. Considering the entire cohort for Study 1 (n=26), specificity and sensitivity were 100% and 94%, respectively. If PanIN samples were excluded, specificity and sensitivity were both 100% (n=21). The Spearman correlation coefficient between percent fibrosis and CRF was $r(15)= 0.82$ ($p<0.001$) which indicates a strong positive correlation. For Study 2 all AP and saline-injected pancreases were diagnosed as non-cancerous. Regression analysis revealed a positive correlation between biomarker and pathologically analyzed cancer-induced fibrosis ($r(24)=0.73$ ($p<0.001$)). In Study 3, the sensitivity and specificity were 83.3% and 87.5% respectively comparing CRF with pathological assessment. CONCLUSIONS: These findings demonstrate CRF as a potential diagnostic biomarker for PC and support NovaScan's CRF technology to decipher between AP and PC. Implementation of the nsCanary device into clinical workflow will support realtime feedback on cancer detection and sample adequacy, preventing additional biopsy procedures. Current work aims to confirm and extend these findings using fine needle biopsies (FNBs) from ex vivo mouse PC samples. Future work will determine if increased CRF values are consistent with more sinister PanIN 3 lesions and if CRF can decipher between PC and chronic pancreatitis (CP).

Poster 21

Ines Pulido Endrino

Overcoming acquired resistance to KRAS(G12D) inhibition using a KRAS-HSP90 hetero-bifunctional small molecule therapeutic agent

ABSTRACT: BACKGROUND, PURPOSE, or OBJECTIVES: KRAS mutations are highly prevalent across many different cancers types. Recent advancements in KRAS-targeted drugs, such as the FDA-approved KRAS(G12C) inhibitors sotorasib and adagrasib for NSCLC patients, have shown great promise in the clinic. Nonetheless, these new agents fail to address other mutations, such as KRAS(G12D), which overall is the most common KRAS mutation in cancers, being found in 37% of pancreatic ductal adenocarcinomas, 12.5% of colorectal cancers and 4.9% of lung adenocarcinomas. Recently, MRTX1133 has been described as a selective, non-covalent inhibitor of KRAS(G12D) that shows promising preclinical efficacy and is undergoing clinical testing. However, based on prior clinical experience with sotorasib and adagrasib, acquired resistance to MRTX1133 may reasonably be anticipated. HYPOTHESIS/AIMS: The main aim of the study is to investigate mechanisms of drug resistance and how to overcome them pharmacologically. MATERIALS or METHODS: We used KRAS(G12D)-mutated cell lines and a patient-derived organoid models that exhibit varying degrees of inherent resistance to MRTX1133. To examine the consequences of blocking KRAS signaling, we employed a KRAS(G12D)-specific PROTAC, which simulated the effects of KRAS deletion. RESULTS: We demonstrate that receptor tyrosine kinase (RTK) activation could compensate for loss of KRAS signaling and was a key de novo resistance mechanism. This suggests that combination therapies may need to be individually tailored to treat patients resistant to KRAS(G12D)-targeted therapies. To counteract this, we employed KRAS(G12D)-CHAMP RNK08179, a hetero-bifunctional small molecule agent that simultaneously targets both KRAS(G12D) and HSP90, an RTK-regulating chaperone protein. CONCLUSIONS: RNK08179 effectively suppressed both KRAS signaling and RTK activation in MRTX1133-resistant cancer models, overcoming the compensatory resistance mechanisms observed with MRTX1133 alone. Our findings highlight the potential of KRAS-CHAMPs as a novel, effective treatment strategy for KRAS-driven cancers, particularly those resistant to KRAS(G12D) inhibitors.

Poster 22

Chih-Jia Chao

Antigen capturing nanoparticle boosted cDC1 therapy for in situ cancer immunization

Antigen capturing nanoparticle boosted cDC1 therapy for in situ cancer immunization ABSTRACT Background: Cancer immunotherapy, which aims to stimulate the immune system to induce antigen-specific immune responses against cancers, has emerged as a promising approach for cancer intervention. However, eliciting an effective antitumor immune response is hindered by the scarcity of appropriate antigen-presenting cells (APCs) and their impaired function within the immunosuppressive tumor microenvironment. This limits the ability to effectively present tumor antigens to immune cells in the lymph nodes. To improve the success of cancer immunotherapy, developing a robust immunotherapeutic technology capable of both modulating the suppressive tumor microenvironment and facilitating tumor antigen presentation remains an urgent unmet need. Aims: Our goal is to develop a novel anti-tumor immunotherapy that overcomes the suppressive tumor microenvironment and facilitates in situ immunization, resulting in efficacious and enduring therapeutic effects. Methods: We employ a Trojan horse strategy leveraging antigen capturing nanoparticles (AC-NPs) and migratory CD103+ type 1 conventional dendritic cells (cDC1s), named Antigen Capturing nanoparticle Transformed Dendritic Cell therapy (ACT-DC). AC-NPs are engineered to capture antigens directly from the tumor and facilitate their delivery to adoptively transferred migratory CD103+ cDC1s, which enhance antigen presentation to the immune cells in lymph nodes and reshape the tumor microenvironment. Results: Our findings demonstrate that ACT-DC could improve in situ antigen collection and increase antigen delivery to lymph node. Additionally, ACT-DC could trigger a strong systemic immune response without the need for exogenous antigens, and transform the tumor environment into a more immunogenic state. In different tumor models including the MC38 and B16F10 models, ACT-DC combined with immune checkpoint inhibitors led to 75-80% tumor-free survival, even after two tumor rechallenges, indicating potent immune response and immunological memory. Conclusions: In this work, we highlight the capability of ACT-DC to enhance in situ immunization for systemic tumor eradication. ACT-DC harness intratumoral antigen, modulates local tumor microenvironment, and improves antigen presentation leading to efficient anti-cancer immunity and long-term tumor rejection. The ACT-DC approach could be a broadly effective strategy for in situ cancer immunization and tumor microenvironment modulation.

Poster 23

Elie Abi Khalil

Tissue region-selective multiomics: a novel cellular and molecular analysis method of the tumor immune landscape

Tissue region-selective single-cell spatial multiomics: a novel method for immune landscape analysis in solid tumors PRESENTING AUTHOR: Elie Abi Khalil CO-AUTHORS: Yi-Chien Wua, Joshua T. Plankb, Alexander Lippertb, Steve Seung-Young Leea a. Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois Chicago b. Department of Chemistry, Southern Methodist University ABSTRACT: BACKGROUND, PURPOSE, or OBJECTIVES: The tumor-immune microenvironment (TIME) is a complex ecosystem consisting of various distinct subregions, where different types of immune cells, cancer cells, and other structural cells interact in unique ways. Accordingly, 'hot' tumors that show a high degree of immune infiltration have been correlated with increased overall patient survival in comparison to 'cold' tumors which are characterized by minimal immune infiltration. Notably, even within infiltrated tumors, while some cell populations such as cytotoxic CD8+ T-cells and M1 macrophages constitute a hallmark of anti-tumor activity, other populations such as regulatory CD4+ T-cells and M2 macrophages seem to carry out protumor functions. It has also been revealed that there are unique molecular profiles that arise from the proximity of immune cells to one another and to the stromal component, namely, cancer associated fibroblasts (CAFs). This functional heterogeneity has encouraged the exploration of the molecular underpinnings governing the phenotypic diversity within the tumor tissue niche. With the recent emergence of spatial omic technologies, we have thus been able to explore these nuances at previously inaccessible resolutions. This comes with a major emphasis on the spatial context associated with the cellular phenotypes of interest- but it comes at the cost of introducing bias, losing molecular information, and incompatibility with single cell technologies. HYPOTHESIS/AIMS: Our goal is to develop a platform for the selection and isolation of tumor cells and infiltrating lymphocytes from user defined regions of the tumor for downstream multiomic and functional analysis. MATERIALS or METHODS: Here, we leverage a photoactivatable (PA) dye Janelia-Fluor-646 in combination with a digital light processing (DLP) microscope to address these issues by developing a platform that enables the spatially guided isolation of cellular populations within a region of interest (ROI) for downstream multiomic and single cell analyses. RESULTS: As proof of concept, we show that ROIs could successfully be barcoded in mouse spleen and tumor sections using the dye and distinguished from the background by fluorescence activated cell sorting (FACS). We also demonstrate the ability to extract good quality DNA, RNA, and protein as well as maintain high cell viability following collection of our resulting cell populations. CONCLUSIONS: Moving forward, we hope to establish this as a platform for deep molecular analysis of the intratumoral immune landscape to uncover unique targets that could be explored within the context of tumor-immune interactions as well as immunotherapeutic development.

Poster 24

Dahee Jung

Engineered anti-CD40 agonist antibody as a novel cancer neoantigen vaccine delivery system

Purpose: Somatic mutations in cancer cells lead to the expression of neoantigens that can be targeted by personalized vaccines. However, current cancer vaccines have shown poor clinical outcomes due to the lack of effective delivery methods. To address the issues, our study aims to develop a novel vaccine platform that targets dendritic cells (DCs) and delivers neoantigen peptides more effectively through the use of an anti-CD40 agonistic antibody expressing monovalent streptavidins (_CD40-mSAs). The agonistic anti-CD40 antibody will target and activate DCs, and the mSA compartment will allow the loading of two biotinylated payloads (e.g. peptides, proteins, RNAs) on each vaccine carrier. Aims: Our goal is to directly deliver neoantigen peptide-loaded _CD40-mSAs to DCs in draining lymph nodes (dLNs) via subcutaneous (s.c.) injection as a therapeutic cancer vaccine. Method: CHO cell-based recombinant protein production method was used to biosynthesize _CD40 and _CD40-mSAs. First, cloning vectors were reconstructed based on mouse IgG2a, incorporating the peptide sequences on the complementarity-determining regions (CDRs) of anti-mouse CD40 agonist antibody (clone FGK45) and monovalent streptavidin. Cloning vectors were transfected to ExpiCHO expression system to produce antibodies. Protein G column was used to purify the produced antibodies. SDS-PAGE assay was conducted to confirm the expression of mSAs in the light chains of _CD40. To evaluate the binding affinity of _CD40-mSAs to mouse CD40, surface plasmon resonance (SPR) was performed. Fluorescence polarization (FP) was used to confirm the biotin-binding property of the monovalent streptavidin compartment. Flow cytometry was employed to confirm the activation effects of _CD40 on bone marrow-derived dendritic cells (BMDCs) after 24h incubation. After subcutaneous (s.c.) injection on the mouse footpad, lymph nodes were retrieved, stained with a dendritic cell marker, and imaged using confocal microscopy to confirm the rapid transport of antibodies to the neighboring lymph node. Results: The study successfully produced _CD40-mSAs, as confirmed by SDS-PAGE gel data showing the increased molecular weight of the light chain of _CD40-mSAs at approximately 40 kDa, indicating the expression of streptavidin monomer structure. Surface Plasmon Resonance(SPR) analysis showed both _CD40 and _CD40-mSAs has high-affinity binding to CD40 and had similar KD (equilibrium dissociation constant) of 12.3 and 12.7 nM, respectively. FP confirmed that FITC-biotin binds with _CD40-mSAs with 111.4 nM KD. Flow cytometry confirmed that the activation markers, such as CD40, CD86, and MHCII, increased after 24h incubation of _CD40 with BMDCs. After collecting a popliteal lymph node (LN) at 15 min post-injection and staining for CD11c+ dendritic cells (DCs), we observed that subcutaneously injected _CD40 rapidly moved into the nearest draining lymph node and bound to DCs. It has been determined that over 44% of the CD11+ DCs' area in the draining lymph node colocalized with subcutaneously injected _CD40. Conclusion: In conclusion, this study developed an effective cancer vaccine delivery platform using _CD40-mSAs to target DCs and deliver neoantigen peptides more effectively. This study provides a promising strategy for delivering personalized neoantigen vaccines and enhancing their efficacy by using an effective carrier.

Poster 25

Celine Macaraniag

VALIDATION OF MICROFLUIDIC ISOLATION OF CIRCULATING PANCREATIC TUMOR CELLS FROM BLOOD

BACKGROUND, PURPOSE, or OBJECTIVES: Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis and is the fourth leading cause of cancer-related deaths in the US. PDAC is diagnosed in the late stages, which limits treatment options and yields poor clinical outcomes. Recurrence is a significant issue for patients undergoing surgery for PDAC. Circulating tumor cells (CTCs) are thought to be a potential mediator of recurrence, facilitating the formation of secondary tumor sites after surgery. Thus, there is a need to isolate CTCs to improve understanding of disease recurrence, to ultimately improve outcomes. HYPOTHESIS/AIMS: We aim to validate our inertial microfluidic (iMF) system for isolating pancreatic CTCs from blood. We also benchmark our iMF system to a commercially available immunomagnetic negative selection system, EasySep™, and quantify target cell recovery and cell enrichment. MATERIALS or METHODS: Our iMF system employs the differential cell sizes of CTCs from blood cells and inertial migration of such cells in a straight microchannel (150 Qm _ 50 Qm _ 24 mm) to enrich CTCs from blood. The iMF system sample flow rate used is 100 QL/min, with a 200 QL/min PBS buffer flow rate. The EasySep™ Direct Human CTC Enrichment kit targets CD2, CD14, CD16, CD19, CD45, CD61, CD66b, and Glycophorin to remove blood cells from the sample using magnetic particles, thereby separating them from CTCs. We spiked low cell concentrations of 50, 100, and 500 Hoechst-stained pancreatic carcinoma cells, PANC1, in 1 mL of healthy blood then captured using either EasySep™ or iMF. Cell enumeration was performed after centrifugation or Cytospin. RESULTS: PANC1 cells and WBCs have average sizes of 18 Qm and 8.4 Qm, respectively. We characterized our iMF device to capture cells ≥12 Qm, which yields capture of most PANC1 cells. Indeed, our iMF system achieved 82.8319.2%, 72.5316.3%, and 59.333.8% recovery for 50, 100, and 500 cell-spikes in lysed blood, respectively. For the same 50, 100, and 500 cell-spikes, the total iMF CTC yields after Cytospin were 46.3311.8, 44.339.1, and 32.732.1%, while the total CTC yields from EasySep™ were 4.633, 5.332.1, 8.331%, respectively. When we processed whole blood directly, EasySep™ had lower cell recovery, 3.830.2, 4.631, and 3.330.4%, respectively. Our system also demonstrated 8_ higher target cell enrichment compared to EasySep™. Blood lysis before iMF separation did not drastically affect the recovery of target cells. CONCLUSIONS: We determined that our system has improved performance over the commercial isolation system, EasySep™, thereby validating the effectiveness of microfluidic isolation for PDAC. In future studies, we can then use our iMF system in CTC isolation from routine blood draws from PDAC patients undergoing surgical resection. We may then isolate real patient samples and study relevant phenotypes of PDAC CTCs which may therefore be used for identifying certain mechanisms of PDAC progression.

Poster 26

Courtney E Ketchum

Extraneural Metastasis of Glioblastoma: A Review of Literature

Background: Extraneural metastases (ENM) from glioblastoma (GBM) represent a rare and poorly understood phenomenon, with limited data available regarding their occurrence rate, risk factors, progression, response to therapy, and impact on survival. Existing literature predominantly focuses on reporting the involvement of different organs, lacking comprehensive analyses of prognostic factors and therapeutic implications. Furthermore, the role of the MGMT promoter methylation status, a highly impactful predictive marker in GBM, remains unexplored in cases of GBM with extraneural metastases. This review aims to address these knowledge gaps by identifying all GBM ENM reported cases and evaluating the role of various patient-specific, treatment-specific, and tumor-specific factors on the survival of this patient population. The findings of this study will provide valuable insights into the prognostic implications of ENM in GBM patients and inform clinical decision-making regarding treatment strategies. Methods: A comprehensive PubMed literature search was conducted to identify all reported GBM ENM cases up to March 2023. The search strategy employed a combination of keywords, including “glioblastoma and metastasis,” “GBM and metastasis,” and “astrocytoma and metastasis.” Data extraction included patient demographics, tumor characteristics, treatment modalities, and survival outcomes. Statistical analysis utilizing Kaplan-Meier analysis and Cox regression modeling were performed to evaluate the impact of various factors on survival. Results: We identified 210 cases including 146 males and 64 females with a median age of 46 years. Most common CNS locations were temporal lobe (29.7%) and frontal lobe (26.9%) while most frequent sites of ENM were bone (30.7%) and lung (20.6%). Median latency from GBM diagnosis to ENM occurrence was 10 months and median OS was 13.5 months. Aggressive multimodal therapy, defined as a combination of surgery, chemotherapy, and radiation, resulted in significantly improved median OS after diagnosis (17 months vs. 9 months; p-value < 0.0001) and post-ENM (4.5 months vs. 2 months; p-value 0.004). Interestingly, the co-occurrence of CNS recurrence with ENM was associated with prolonged median OS (17 months vs. 13 months; p-value 0.04). There was no statistically significant impact on survival based on gender, age, metastatic burden or MGMT promoter methylation. Conclusions: Our study represents the most comprehensive analysis of GBM ENM, shedding light on the occurrence and prognosis of this rare phenomenon. Through meticulous review of 210 cases, we have provided updated insights into patient demographics, tumor characteristics, treatment modalities, and survival outcomes. The findings reinforce the importance of aggressive multimodal therapy as it improved both OS and post-ENM OS. We also show that unlike its impactful role in GBM, MGMT promoter methylation did not improve the survival of GBM patients with ENM. Moreover, our study underscores the pivotal role of the tumor microenvironment, particularly the immune system, in GBM pathogenesis. The low rate of GBM ENM may reflect the robustness of systemic immunity compared to CNS immunity. By consolidating available evidence and highlighting areas requiring further investigation, this review contributes to a better understanding of GBM ENM and informs future research efforts aimed at improving patient outcomes and personalized therapeutic strategies.

Poster 27

Yi-Chien Wu

Tissue-niche-based and cell-type-selective proteomics

Background: Proteins are the fundamental components of cellular functionality, governing a myriad of physiological processes. The intricate spatial organization of cell populations within tissues and organs profoundly influences normal physiological function and the pathogenesis of various diseases. Spatial proteomics has emerged as a crucial field for comprehensively understanding the mechanisms underlying tissue homeostasis, disease progression, and therapeutic responses by integrating information on cellular location and molecular profiling. Laser microdissection coupled to mass spectrometry (LCM-MS) is a widely used spatial proteomics approach that applies an ultraviolet laser to dissect regions of interest from the tissue, followed by protein profiling on a mass spectrometer. However, due to the laser beam thickness and cell burning issue, space offset away from the target cells always results in protein contamination from the cell surroundings. Besides, it requires researchers' meticulous effort to delineate and dissect individual cells to collect specific cell populations for cell-type-selective protein analysis. The process is time-consuming, mainly when working on small cells in dense regions (i.e., immune cells in the spleen) and irregular cells (i.e., neurons and endothelial cells). To address these challenges, we introduced a novel spatial proteomics method enabling targeted protein analysis of specific cell phenotypes within defined tissue niches. Methods: Our spatial proteomics approach incorporates immunofluorescence (IF) staining, microscopic photobleaching, fluorescence cell sorting, and liquid chromatography-mass spectrometry (LC-MS) analysis in a subsequent pipeline. Tissue macrosections around 400-Qm-thick were stained with fluorescence-conjugated antibodies to visualize specific cell types of interest. We employed two fluorescence-conjugated antibodies of the exact clone (e.g., Alexa488-anti-CD11c and Alexa647-anti-CD11c antibodies) for IF staining on the interesting cell population (e.g., CD11c+ cells). We then introduced "photobleaching barcodes" using a confocal microscope to exhaust fluorescence signals stained on cells by exposing them to the matched laser. Photobleaching changes the fluorescent signal on the illuminated cells and creates a differentiable fluorescence barcode that records their spatial information. After tissue dissociation, barcoded cells were sorted into groups and classified by their original locations in the tissue macrosections. Lastly, protein extraction and LC-MS analysis were conducted on the collected cells to enable comprehensive spatial proteomic analysis. Results: The initial application in investigating dendritic cell (DC) subsets in mouse spleen during lipopolysaccharide (LPS)-induced inflammation reveals significant proteome differences among three splenic DC subsets, categorized by their locations in or outside spleen T-cell zones as well as control DCs. Our result aligns with previously published proteome data in splenic DCs, demonstrating the feasibility and reproducibility of our technology. Furthermore, we anticipate adapting this technology to analyze tumor samples, including mouse peripheral T cell lymphoma and human breast tumors, to evaluate tumor cell heterogeneity and to profile the tumor-immune microenvironment. Conclusions: This technology features broad protein profiling for cell-type-informative spatial proteomics and is designed for extensive applications across diverse tissue specimens in various diseases. Spatial proteomic findings from both applications are poised to identify new cell types and discover potential drug targets that benefit treatment efficacy for inflammatory diseases and cancer.

Poster 28

Luke N Redlon

Development of liver cancer cell models by gene alteration in primary porcine hepatocytes

Development of liver cancer cell models by gene alteration in primary porcine hepatocytes Abstract Background: Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide with a low 5-year relative survival rate of 21.6%. HCC continues to rise in prevalence with the increase in risk factors such as obesity, diabetes mellitus, excessive alcohol consumption, and hepatitis B/C diagnosis. With limited availability of cell and animal models that accurately represent the characteristics of human HCC, the goal of this project is to develop tumorigenic models using hepatocytes isolated from wild type pigs. Porcine models more accurately recapitulate human diseases due to significant similarities in drug sensitivity, efficacy, and toxicity. As large animals with longer lifespans compared to murine models, porcine models of HCC would be an invaluable tool for interventional radiology procedural applications. The Barcelona clinic liver cancer staging system currently directs treatment of HCC, and when resection is not indicated, a one size fits all medication regimen is the current best practice. The development of representative tumor models with a defined mutation profile, will improve our ability to identify precision therapies based on specific patient tumor mutations. Methods: Hepatocytes were isolated from Yucatan minipigs (n=3) and subsequently underwent genetic mutation via clustered regularly interspersed short palindromic repeats (CRISPR) and CRISPR associated protein 9 (CAS-9), with or without lentiviral transduction of c-myc. Two cell lines of interest were developed in this manner from each animal. The cells were developed by introducing the following combinations of gene mutations: 1) TP53 knockout (KO), PTENKO, and c-myc overexpression (OE), referred to as TPM, and 2) TP53KO, CDKN2AKO, and c-mycOE, referred to as TKM. This approach resulted in heterogeneous cell populations with varying gene edits and c-myc expression levels in individual cells. The cells were grown until the mutations, which confer a selective growth advantage, were enriched. CRISPR induced gene edits were validated by Sanger sequencing followed by analysis with Synthego inference of CRISPR edits (ICE) and c-myc integration was validated by PCR. Each cell line underwent DNA extraction and PCR/Sanger sequencing analysis at regular intervals. Finally, these cell lines were injected subcutaneously into severe combined immunodeficient (SCID) mice to evaluate their tumorigenicity and histological features. Results: Consistent with their known tumor suppressor roles, analysis of TP53, PTEN, and CDKN2A edits demonstrated a progressive enrichment of KO mutations in the cultured cells. PCR analysis confirmed the integration of transduced c-myc. Injection of cells with enriched KO mutations into SCID mice resulted in tumor development, confirming the tumorigenicity of the developed cells. Histological analysis demonstrated these masses to be epithelial neoplasms resembling human HCC. Conclusions: CRISPR induced KO mutations of TP53, PTEN and/or CDKN2A combined with c-myc overexpression in porcine hepatocytes confers a selective growth advantage, and results in tumorigenicity confirmed by tumor development in SCID mice. These innovative genetically defined HCC models are valuable tools for mechanistic studies and high throughput drug screening. Further, these models provide rationale for the development of large animal models suitable for various interventional oncology therapies by autologous cell transplant into pigs.

Poster 29

Sanjay S Ganesh

Automated Segmentation and Classification for Early Detection of Uveal Melanoma

BACKGROUND: Uveal melanoma (UM) is the most common intraocular malignancy in adults and is known to have a poor prognosis. Accurate diagnosis of UM is challenging as these tumors closely resemble various other intraocular lesions. Though benign, it is also important to identify and monitor these lesions, as early detection of malignant transformation into UM has the potential to improve patient outcomes. Current screening and triaging methods for melanocytic choroidal tumors face inherent limitations, particularly in regions with limited access to specialized ocular oncologists. Machine learning (ML) provides a promising avenue for automating tumor identification. Though ML-powered segmentation has been demonstrated in other areas of ophthalmology, it has not yet been shown in the context of UM and its mimickers. AIM: This study explores the potential of ML to automate tumor segmentation, focusing on enhancing screening techniques for choroidal tumors using ultra-widefield fundus photography. Images were obtained at the time of initial presentation from patients diagnosed with UM, choroidal nevi, or congenital hypertrophy of the retinal pigmented epithelium (CHRPE). The primary objective was to develop an ML model capable of accurately segmenting lesions to aid in detecting areas of interest within each image. Additionally, the model was trained to classify images into one of four categories: UM, nevi, CHRPE, or healthy. The study evaluated the overall segmentation and classification performance to assess its efficacy. METHODS: A retrospective chart review was conducted of patients diagnosed with UM, choroidal nevi, or CHRPE at a tertiary academic medical center. Patients included had a single ultra-widefield fundus photo (Optos PLC, Dunfermline, Fife, Scotland, UK) of adequate quality to visualize the lesion of interest as confirmed by a single ocular oncologist (MJH). These images were used to develop and test an ML algorithm for lesion segmentation. RESULTS: A total of 396 images were used to develop an ML algorithm for lesion segmentation. 90 additional images were used in the testing dataset, along with 30 healthy control images. Performance was measured using Dice coefficients with values closer to 1 indicating a greater degree of similarity. Of the images with successfully detected lesions, the ML segmentation yielded Dice coefficients of 0.86, 0.81, and 0.85 for UM, choroidal nevi, and CHRPE, respectively. Sensitivity for any lesion detection per image was 1.00, 0.90, and 0.87 respectively. For images without lesions, specificity was 0.93. CONCLUSIONS: Automated lesion segmentation via ML provides an effective screening method that may be accessible to providers globally, irrespective of the availability of specialists. This ML model delivers accurate lesion delineation to assist in tumor identification and improve the interpretation of ophthalmic imaging. Future directions include investigating the enhancement of lesion classification within the model and further exploring its effectiveness in improving diagnosis and patient outcomes.

Poster 30

Ayesha Khan

Reduction in NSCLS tumorigenesis by targeting Sphingosine Kinase 2

Background: Lung cancer will account for 20% of US cancer deaths in 2024 with over 200,000 new cases and 125,000 plus projected deaths. Treatments that help regulate cell growth, repair and survival, such as EGFR-TKi (epidermal growth factor receptor tyrosine kinase inhibitors), initially decrease tumor growth, but often leads to TKI resistance. According to studies, non-small cell lung cancer (NSCLC) tumors express more SphK2 (Sphingosine kinase 2) than normal lung tissue, and patients with overexpression have worse survival chances. SphK2 promotes cancer progression by interacting with other proteins. However, SphK2's role in TKI resistance is unclear. Hypothesis/Aims: We hypothesize that inhibiting SphK2 could reduce tumor progression and overcome resistance to lung cancer drugs such as ER (Erlotinib) and OR (Osimertinib). However, patients become tolerant towards them due to TKI resistance. Methods: We studied the expression of SphK2 in both drug-resistant (OR/ER) and drug sensitive (parental) NSCLC cell lines using techniques that detect SphK2 at the protein, DNA and tissue levels. NSCLC cells were also bioprinted with bioink to mimic the physiological tumor environment and treated with SphK2 RNA inhibitor. We also performed cell-based assays to analyze the metastatic properties of OR resistant cells. Results: Protein analysis was done to detect changes in the expression of SphK2 in parental and OR-resistant cell lines. Results indicated a 1.5-1.8-fold upregulation of SphK2. Increased SphK2 gene expression was observed in TKI-resistant cell lines (4.3-fold), with a 1.8-3.3 fold overexpression compared to mutant EGFR and a 1.3-1.5 fold overexpression compared to 'normal' EGFR parental cells. Fluorescence based studies showed increased fluorescence of SphK2 in different NSCLC cell lines by 2.6-fold, 2.4-fold and 2.3-fold compared to parental cells. Once we knew that blocking SphK2 might overcome oncogenesis, we checked if reducing SphK2 could reverse the invasive process called Epithelial-Mesenchymal Transition (EMT). After knocking down SphK2 with interfering RNAs, gene expression of SphK2 was downregulated by 3.8-fold compared to control, and a number of EMT biomarkers were also downregulated 5-fold and 3.3-fold. Spheroids formed from single cancer cells can "re-create" the growth of a tumor. We used 3D-bioprinting to precisely stack cells on biological scaffolds to recreate lung tumors from patients. The number of bioprinted spheroids of NSCLC cells treated with SphK2-I and OR alone were compared to a combination of OR and SphK2-I. The inhibition of spheroid formation was 87.3% compared to cells treated with OR alone, and 78.2%compared to cells treated with SphK2-I alone. Bioprinted spheroids in yet another NSCLC cell line treated with SphK2 inhibitor and OR revealed a 75.8% and 81.4% reduction of spheroid count, respectively, compared to treatments with inhibitor alone. For the cell-based assays, treatments with the drug OR and inhibitor resulted in a decrease in spheroid formation by 56% and 73.98%. In a second cell based assay, SphK2-I and OR inhibited wound closure by 90.48% compared to diluent and 86.68% compared to OR ($p<0.001$). Conclusion: These results suggest that inhibiting SphK2 reverses the migratory characteristics of NSCLC cells and potentially overcomes Osimertinib resistance, which supports our hypothesis.

Poster 31

Sabrina P Iddir

Predicting Malignant Transformation of Choroidal Nevi Using Machine Learning

Background Uveal melanoma (UM) is the most common intraocular malignancy in adults, with a high rate of metastasis and a poor prognosis. The accurate diagnosis of small UM is challenging due to similar clinical characteristics to benign choroidal nevi. Tumors diagnosed as choroidal nevi that subsequently grow during an observation period are at increased risk for metastasis. Therefore, improving the diagnosis of UM and choroidal nevi at the time of initial presentation has the potential to improve clinical outcomes. This study aims to assess a machine learning (ML) algorithm using multimodal imaging to accurately identify risk factors for UM and aid in the diagnosis of melanocytic choroidal tumors. Methods This study included 223 eyes from 221 patients with melanocytic choroidal lesions seen at the eye clinic of the University of Illinois at Chicago between 01/2010 and 07/2022. An ML algorithm was developed and trained on ultra-widefield fundus imaging and B-scan ultrasonography to detect risk factors of malignant transformation of choroidal lesions into UM. The risk factors were verified using all multimodal imaging available from the time of diagnosis. We also explore classification of lesions into UM and choroidal nevi using the ML algorithm. Results The ML algorithm assessed features of ultra-widefield fundus imaging and B-scan ultrasonography to determine the presence of the following risk factors for malignant transformation: lesion thickness, subretinal fluid, orange pigment, proximity to optic nerve, ultrasound hollowness, and drusen. The algorithm also provided classification of lesions into UM and choroidal nevi. A total of 115 patients with choroidal nevi and 108 patients with UM were included. The mean lesion thickness for choroidal nevi was 1.6 mm and for UM was 5.9 mm. Eleven ML models were implemented and achieved high accuracy, with an area under the curve of 0.982 for thickness prediction and 0.964 for subretinal fluid prediction. Sensitivity/specificity values ranged from 0.900/0.818 to 1.000/0.727 for different features. The ML algorithm demonstrated high accuracy in identifying risk factors and differentiating lesions based on the analyzed imaging data. Conclusions This study provides proof of concept that ML can accurately identify risk factors for malignant transformation in melanocytic choroidal tumors based on a single ultra-widefield fundus image or B-scan ultrasound at the time of initial presentation. By leveraging the efficiency and availability of ML, this study has the potential to provide a non-invasive tool that helps to prevent unnecessary treatment, improve our ability to predict malignant transformation, reduce the risk of metastasis, and potentially save patient lives.

Poster 32

Sakshi V Bansod

Investigating the Carcinogenic Effects and the Mechanism of Cadmium Exposure on WPMY-1 Prostate Myofibroblast Cell Line.

I BACKGROUND, PURPOSE, or OBJECTIVES: Prostate cancer (PCa) is the second most common cancer among males with an incidence rate of 288,300 new cases annually. One potential emerging cause of PCa is exposure to metal carcinogens such as cadmium (Cd) which is now classified as a group I human carcinogen. Chronic exposure to Cd on epithelial cells has been extensively shown to induce carcinogenesis, making it a likely contributor to PCa development. However, the carcinogenic effects of Cd on prostate fibroblast cells are not well known. This study explored how chronic Cd exposure affects prostate-derived myofibroblast stromal (WPMY-1) cells transforming into a cancer-associated fibroblast-like phenotype. HYPOTHESIS/AIMS: We hypothesize that chronic exposure to cadmium can transform prostate myofibroblasts into malignant cancer-associated fibroblast-like phenotype. Hence, the cadmium-transformed myofibroblasts will promote cancer progression. MATERIALS or METHODS: To study the malignant transformation, WPMY-1 cells were treated with 500nM of Cd for over 11 months establishing the WPMY-1 Cd cells, WPMY-1 and CAF cells were used as control cell lines. To understand the phenotypic characteristics in Cd transformed WPMY-1 cells, an in-vitro 2D- colony formation assay and 3D spheroid assay was performed to ascertain the property of clonal expansion of WPMY-1 Cd. To determine whether carcinogenic effect of Cd on the WPMY-1 promotes migration property of WPMY-1 Cd wound healing assay was performed. To screen the effects of Cd exposure at molecular level, expression of CAF markers was analyzed in WPMY-Cd cells using western blotting. CAFs play a role in PCa progression by promoting tumor growth via the secretion of cytokines and growth factors. The theory of stromal cell promoting cell migration was tested by treating Cd-transformed epithelial (BPH-Cd) cells to conditioned media containing secretions from CAF, WPMY-1, and WPMY-Cd via wound healing assay. Additionally, BPH-Cd spheroids were treated with stromal cell conditioned media to understand whether stromal cell interaction promotes the property of cell proliferation and clonal expansion. CD90 and CD44 stem cell marker were analyzed using flow cytometry. RESULTS: Our data suggests that WPMY-Cd cells formed dense spheroids and exhibited sprouting characteristics. Formation of invadopodium is a property of invasive characteristics aiding in ECM remodeling. WPMY-1 Cd cells formed a greater number of colonies suggesting enhanced cell proliferative property. CAF markers such as vimentin, FAP, _-SMA, HSP47, HSF1, and PDGFR-_ for WPMY-Cd cells showed a distinct upregulation in expression profile when compared to untreated WPMY-1 cells, suggesting that chronic exposure to Cd promotes CAF-like phenotypic transformation in WPMY-Cd. BPH-Cd and LNCAp cells treated with conditioned media of WPMY-1 Cd and CAF led to complete wound closure in 48 hours compared to WPMY-1 cells depicting the pro-migratory effects of the conditioned media. BPH-Cd cells formed greater spheroids when treated with WPMY-1 Cd-conditioned media as compared to WPMY-1 and CAF CONCLUSIONS: Overall, there is considerable evidence that Cd exposure in fibroblasts may play a crucial role in PCa disease progression and is a promising area for researching PCa treatment strategies against a crucial component of the tumor microenvironment.

Poster 33

Manoela Lima Oliveira

Design of a Remote Time-Restricted Eating and Mindfulness Intervention to Reduce Risk Factors Associated with Early-Onset Colorectal Cancer Development among Young Adults

Background: Early-onset colorectal cancer (EOCRC) is defined as a diagnosis of colorectal cancer (CRC) in individuals younger than 50 years of age. While overall CRC rates in the United States (US) decreased between 2001 and 2018, EOCRC rates have increased. EOCRC shares many risk factors with CRC, including excess adiposity, diet quality, and microbiome dysregulation. Obesity early in adulthood that persists with aging is strongly associated with an increased risk of EOCRC. This is a concern since obesity affects 40% of young adults (20-39 years of age) in the US. Stress may also increase the risk of EOCRC. The prevalence of perceived psychosocial stress in young adults varies widely, with studies reporting rates ranging from 20%-60%. Chronic psychosocial stress adversely affects several systems of the human body, with implications for tumorigenesis. Objective: To evaluate the feasibility and acceptability of an 8-week remote intervention in Time-Restricted Eating (TRE), Mindfulness, or TRE combined with Mindfulness among young to middle-aged adults (18-39 years of age) at risk of EOCRC development. Methods: Forty-eight participants will be randomly assigned to one of four groups: TRE, Mindfulness, TRE and Mindfulness, or Control. Participants randomized to the TRE intervention group will be instructed to eat ad libitum from 12:00-8:00p.m. daily and fast during the remaining hours (16-h fast). Participants randomized to the Mindfulness group will be granted access to the Calm.com platform. Participants will have access to the “Mindfulness for Beginners” course, consisting of 30 audio lessons, each ranging from 9 to 14 minutes long. During the study, participants will be asked to complete four sessions per week during weeks 1-7 and two lessons during week 8. Participants randomized to the TRE and Mindfulness will follow a combined protocol of the TRE and Mindfulness as described above. The Control group will not receive any of the interventions described. All participants will be asked to maintain their baseline level of physical activity throughout the intervention. Data on feasibility, adherence, and acceptability will be collected throughout the intervention. Measures assessed at baseline and post-intervention will include body weight, body composition, dietary intake, physical activity, sleep behavior, circulating biomarkers (glycated hemoglobin, serum glucose, serum insulin, serum tryptophan, and tryptophan metabolites), blood pressure, heart rate, heart rate variability, hair cortisol, and the gut microbiome. Feasibility and acceptability rates will be calculated, and inferential statistics will be conducted to explore potential intervention effects. Data will be managed using REDCap, and analysis will be performed using SAS software. Expected Results: The effects of the intervention on the following will be examined: (1) acceptability and feasibility; (2) body weight, body composition, and adherence to TRE; (3) circulating metabolic, inflammation, and oxidative stress biomarkers; (4) intestinal inflammation; and (5) the gut microbiome. Conclusion: TRE, combined with Mindfulness, holds promise for stress reduction and weight management among individuals at risk of EOCRC. The results of this pilot study will inform the design and development of larger trials aimed at preventing risk factors associated with EOCRC.

Poster 34

Kate E Cares

Diet and Exercise Interventions in Pediatric Cancer Survivors to Address Markers of Inflammaging: A Systematic Review

Background. The majority (99%) of pediatric cancer survivors (PCS) will experience significant chronic disease burden by the age of 50. The development of chronic disease within this population occurs decades earlier than the non-survivor population and studies indicate that PCS have chronic, systemic inflammation after treatment, resulting in accelerated biological aging, also called “inflammaging.” Cardiometabolic risk and accelerated cellular aging due to chronic inflammation can potentially be mitigated by lifestyle changes such as diet and exercise that improve these modifiable risk factors. Objective. To identify diet and exercise interventions in PCS with outcomes directly related to inflammaging such as markers of systemic inflammation or cellular aging and related indirectly through cardiometabolic risk factors and gut microbiome changes. Methods. This systematic review of studies was conducted using the PRISMA guidelines. A search was conducted in Medline (Pubmed), CINAHL and clinicaltrials.gov for randomized and non-randomized controlled trials that included a diet or exercise component with a participant population that included survivors of a childhood cancer. Covidence software was used for the deduplication process as well as screening, data extraction and risk of bias assessment. Two reviewers independently screened titles and abstracts for study inclusion, conducted data extraction and risk of bias assessment. Conflicts were resolved collaboratively. Results. The study search conducted in September of 2023 yielded 208 studies, 12 of which were identified as relevant for data extraction. Two additional studies were identified through NCBI’s monthly email alert of new publications using the original search criteria for a final total of 14 studies. Two papers detail data from the same clinical trial with different parameters. Only one trial had a dietary component alone while most of the studies employed a dietary and exercise intervention (n=7) or an exercise component alone (n=5). No studies to date that fit the inclusion criteria have measured markers of accelerated cellular aging. Two trials measured C-reactive protein, a measure of systemic inflammation, with neither intervention producing significant changes. One study measured absolute neutrophil count and found an increase after a bout of acute exercise in a within group analysis. Few studies produced significant changes in cardiometabolic risk however, improvement in waist to hip ratio (n=2), fasting insulin (n=2) and HOMA-IR (n=2) were observed in within group analysis or repeated measures analysis for exercise interventions while an increase in lean mass in a within group analysis was observed for exercise and diet (n=2). Conclusion. Inflammaging due to anti-cancer treatments may be leading to premature cellular aging and chronic disease in PCS. Diet and exercise interventions may mitigate cardiometabolic perturbances that exacerbate the inflammaging process. More studies that contain a diet component alone and have outcomes directly related to cellular aging and systemic inflammation are needed to better understand how these lifestyle changes can impact and reduce chronic disease risk in PCS.
Funding source. College of Applied Health Sciences Pilot Funding Award

Poster 35

Ifeanyi (Beverly) Chukwudozie

Examining racial differences in area-level factors associated with prostate cancer stage at diagnosis: A systematic review

Examining Racial Differences in Area-level Factors Associated with Prostate Cancer Stage at Diagnosis: A Systematic Review PRESENTING AUTHOR: Ifeanyi Beverly Chukwudozie, MBA, MPH1,2 CO-AUTHORS: Elle Martell, MPH1; Ravneet Kaur, DrPH, MBA3; Rosie Hanneke, MLS4; and Vincent L. Freeman, MD, MPH1 1University of Illinois Chicago, School of Public Health; 2University of Illinois Cancer Center; 3University of Illinois Rockford, College of Medicine; and 4University of Illinois Chicago, Library of the Health Sciences. ABSTRACT: BACKGROUND, PURPOSE, or OBJECTIVES: In the United States, prostate cancer is the most diagnosed cancer and the second highest cause of cancer deaths in men. Despite the high survival rate for prostate cancer compared to other cancers, racial and ethnic disparities persist. Black males are more likely to be diagnosed at an advanced stage, with more aggressive forms, and have two-fold higher age-adjusted mortality rates than non-Hispanic White males. Research studies show that the stage at diagnosis is a critical prognostic factor for survival and access to quality health care. There is increasing evidence of an association between neighborhood area-level contextual factors (e.g., social and physical environments) and prostate cancer stage at diagnosis, but very few systematic reviews. This systematic review examined White and Black racial differences in the association between area-level factors and prostate cancer stage diagnosis in the United States. MATERIALS or METHODS: We systematically obtained relevant peer-reviewed articles published in English until August 30, 2022, from three databases PubMed, Embase, and Scopus. The relevant study included prostate cancer observational studies in adult men (18 years and older) conducted in the United States that measured one or more area-level factors at the level of a geographic unit (e.g., census tract, block group, and zip codes). The systematic review followed the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2020 statement. We used the National Institutes of Health (NIH) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to review the internal validity of the final studies. RESULTS: The reviewers identified 41 articles with a stage at diagnosis as an outcome, of which 11 articles (26%) statistically examined White-Black differences for the association between stage at diagnosis and area-level factor. Most of the final 11 studies (73%) used advanced stage (regional, distant/metastatic stage) as the primary indicator variable, and two studies looked at two different outcomes (e.g., localized/early stage and tumor aggressiveness). The observed area-level factors were grouped as social (62.5%), physical/built environment (18.8%), and health service determinants (18.8%). Neighborhood socioeconomic status (SES) or deprivation composite scores (12.5%) and poverty (12.5%) were the most area-level factors reported in the studies. There were differences in area-level factors associated with prostate cancer severity for Black and White males. Overall, the results showed that Black men were more likely to be diagnosed with advanced-stage or aggressive tumors if they resided in areas with low neighborhood SES, low environmental quality, and the highest deprived and racially segregated areas compared to Whites. CONCLUSIONS: The study summarized results from individual studies exploring the role of neighborhood area-level factors and prostate cancer stage diagnosis in the United States.

Poster 36

Sydney M Olszewski

Exploring Patient Demographics and Completion of Genetic Counseling Referrals

BACKGROUND, PURPOSE, or OBJECTIVES: Hereditary cancer syndromes comprise approximately 10% of all cancers. Evidence-based guidelines recommend that individuals identified as high-risk for hereditary cancer undergo genetic counseling and testing. However, despite United States Preventive Services Task Force recommendations, many patients are not receiving cancer genetic services (CGS), thus missing critical opportunities for detection, action, and the chance to make informed decisions about their health. To design and implement interventions to alleviate disparities in genetic counseling access, the patient population lost to follow up must be characterized. HYPOTHESIS/AIMS: The aim of this project is to characterize the patient population lost to follow up. We hypothesize that there will be differences in gender, race, ethnicity, or age for patients lost to follow up for CGS compared to patients not lost to follow up. MATERIALS/METHODS: Data was obtained from the Mile Square Family Cancer History (MiFamCan) Registry, which is collected as part of a quality improvement initiative that involves administration of hereditary cancer risk assessments (HCRA) to adult patients (aged ≥ 25) in primary care settings at 2 clinic sites in Chicago, IL. Clinic sites were part of the Mile Square Health Center (MSHC), a system of federally qualified health centers. Patients receiving HCRA between August 2021 and November 2023 were included. Patients meeting National Comprehensive Cancer Network (NCCN) eligibility criteria for genetic counseling per their HCRA were directed to no- or low-cost CGS. RESULTS: Of 1242 patients screened, 879 (71%) identified as female, and 628 (51%) were under the age of 50. Additionally, 644 (52%) identified as Black or African American, 448 (36%) as Hispanic or Latino, and 94 (8%) as White. Of all the patients screened, 244 (20%) were identified as high-risk for having hereditary cancer syndrome. Of the 78 high-risk patients that did receive CGS, 64 (82%) identified as female; 73 (44%) were under the age of 50; 38 (50%) identified as Black or African American, 27 (35%) as Hispanic or Latino, and 12 (16%) as White. Of the 166 patients (68%) that did not receive CGS, 118 (71%) identified as female, and 73 (44%) were under the age of 50. Furthermore, 97 (58%) identified as Black or African American, 56 (34%) as Hispanic or Latino, and 10 (6%) as White. CONCLUSIONS: Nearly 70% of patients at high risk for hereditary cancer are lost to CGS follow-up. There are no significant differences in gender, race, ethnicity, or age between the patients who followed up with CGS and those that did not. This suggests factors beyond demographic characteristics may influence patient engagement with CGS follow-up, warranting further investigation into potential barriers and facilitators. Possible factors contributing to the lack of follow-up could include patient understanding of the implications of their genetic risk, logistical barriers to accessing genetic services, and psychosocial factors such as fear or denial. Future research should identify and address these factors to enhance follow-up rates and ensure high-risk individuals receive timely and appropriate genetic counseling & testing.

Poster 37

Kaitlin Chakos

Time restricted eating with and without a Mediterranean diet during chemotherapy for breast cancer

BACKGROUND, PURPOSE, or OBJECTIVES: Current standard care during treatment for breast cancer encourages enough energy intake to avoid lean mass loss, yet most patients struggle with weight gain during treatment. Time restricted eating (TRE) may garner positive effects on treatment outcomes as well as beneficial effects on glucose regulation and body composition. One potential drawback to TRE is that it does not address deficiencies in diet quality. Like TRE, a Mediterranean Diet (MedDiet) may provide benefits to this patient population. Thus combining TRE with a MedDiet may have important health implications for breast cancer patients. TRE alone or combined with a MedDiet in breast cancer patients during chemotherapy treatment is limited or not yet investigated. MATERIALS or METHODS: We describe a 12-week pilot trial of 8-hour TRE, 8-hour MedTRE, or Control among 45 females initiating chemotherapy for Stage I-III breast cancer. HYPOTHESIS/AIMS: Aims of the study include, (1) Feasibility and accessibility and (2) preliminary efficacy on treatment related outcomes and (3) preliminary efficacy on glucometabolic and cancer-related biomarkers, body weight, and body composition. CONCLUSIONS: The long-term goal of this line of inquiry is to improve quality of life and long-term health outcomes to lead to a fully powered efficacy trial of TRE and MedTRE in this patient population.

Poster 38

Madeleine W Fine

First Steps to miRNA-Based Oral Tumor Classifier Validation

BACKGROUND: RNA-based diagnosis and prognosis of oral squamous cell carcinoma (OSCC) has been slow to come to the clinic. Because an early diagnosis of OSCC plays a crucial role in improving a patient's prognosis, and the current method of diagnosis with 90% accuracy requires the patient to visit an oral surgeon at the request of their dentist to receive a scalpel biopsy and histopathology, a noninvasive yet precise method of detecting OSCC is necessary. Benign and malignant oral lesions can have similar presentations in the mouth, making it difficult for dentists to identify and diagnose OSCC in a timely manner, especially because they may often focus on issues with the teeth rather than soft tissue and are not qualified to perform diagnostic surgical biopsies for possible malignancies. AIMS: We explored non-invasive brush biopsy based acquisition of RNA from suspicious oral lesions in order to identify malignant OSCC from benign lesions. Our objective was to create and validate a classifier to differentiate OSCC from oral lichen planus (OLP) based on miRNA measurement. METHODS: A classifier to differentiate OSCC from benign OLP of 93% accuracy was created by considering measurements of over 350 miRNAs using standard high feature analysis with polyadenylation-based RT-PCR miRNA analysis and machine learning. Next, single target RT-PCR was performed for the 14 miRNAs, which contributed maximally to the classifier to verify their utility as OSCC markers. Surprisingly, only 6 markers survived this test as being differentially expressed in OSCC versus OLP, though these 6 were still able to correctly classify OLP and OSCC with >90% sensitivity and 65% specificity for OSCC. Finally, to perform validation, samples were obtained from a second clinician at a different clinic. RESULTS: These showed overall much lower yields and were accurately identified 70% of the time. This less-than-ideal result suggests: 1. Limitations exist with the creation of a classifier based on high feature analysis without technical replicates. 2. Usage of low number of OLPs in training set is not ideal. 3. Usage of different brush biopsy techniques by validation clinician is important. With low yield samples (10 to 200x less than average sample), classification is often wrong. CONCLUSIONS: To create a more accurate classifier, we will expand the catalog of OLP samples to 30 for the training set versus the 50 cancer samples on hand. We will also use a second method to perform high feature miRNA analysis to better vet marker miRNAs, and we will standardize sample collection among clinicians.

Poster 39

Nyahne Bergeron

Leveraging African American Women as Change Agents to Reduce Obesity-Related Breast Cancer Risk: An Evaluation of a Network-Based Pilot Intervention Promoting the Mediterranean Diet

BACKGROUND: Obesity is a key risk factor for breast cancer incidence, recurrence, and mortality among post-menopausal African American women. Many older African American women are likely to report fewer healthy dietary behaviors. Overweight and obese women are likely to share similar dietary patterns as their social counterparts and compared to other racial/ethnic groups, African American women are more likely to receive information about breast cancer risk through their social networks. Thus, African American women who share information on healthy behaviors may become change agents in their networks. This approach presents an opportunity to increase the impact of evidence-based interventions focused on reducing obesity-related breast cancer risk. The purpose of this study was to assess the impact of the MedDiet-Social Networks pilot intervention among study participants who are trained as change agents to improve obesity-related breast cancer risk reduction behaviors and evidence-based information sharing for themselves and their social networks.

Poster 40

Soeun Kang

Hexokinase 2 regulates anti-tumor functions of CD8+ T cell via reprogramming glucose metabolism

One key hallmark of aggressive cancers is their heightened ability to metabolize glucose, providing cancer cells with a competitive advantage over normal cells. This enhanced glycolysis is typically achieved through the upregulation of hexokinases (HKs), particularly HK2, in cancer cells. This positions HK2 as a promising target specific to cancer cells. However, the role of HK2 in the tumor microenvironment (TME) has been understudied. Here, we demonstrate that the cell autonomous deletion of HK2 could inhibit tumor growth by modifying the TME in in vivo mouse models. Cell autonomous HK2 deletion increases the number of CD8+ T cells in the TME of breast tumors developed in MMTV-PyMT mice, whereas inducible expression of HK2 reduces it. As HK2 deletion reduces glucose utilization in cancer cells and metabolic competition in the TME determines tumor outcome, we hypothesized that CD8+ T cells in the HK2 KO TME would experience greater accessibility to glucose. We also hypothesized that the enhanced glucose utilization directly regulates T cell effector function and reprograms their metabolism to fuel enhanced proliferation. Notably, CD8+ T cells derived from HK2 KO TME exhibit enhanced proliferation and cytotoxicity, accompanied by higher glucose uptake. Together, this suggests that HK2 expression in cancer regulates the anti-tumor functions of cytotoxic CD8+ T cells in the TME via reprogrammed glucose metabolism mediated by metabolic competition. Our findings unveil previously unexplored roles of HK2 in the immunosuppressive TME, which will lay the groundwork for and effective HK2-targeted cancer therapy that can synergize with current chemotherapies and cancer immunotherapies

Poster 41

Al Rabee Kassis

Isoform selective HDAC8 inhibitor (OCH3) shows potency in selectively targeting primary AML cells and linking its effect to a common pathway

The treatment outcomes for patients diagnosed with acute myeloid leukemia (AML) are still dismal. Recent advances in understanding AML indicate that the lack of efficacy is primarily due to non-specificity of currently used chemotherapeutics targeting both leukemic stem/progenitor cells (LSC) and normal hematopoietic stem cells (HSC). Thus, a critical barrier is the identification of innovative therapies that selectively target LSC. Histone deacetylase 8 (HDAC8) has been shown to enhance p53 protein deacetylation, which results in inactivation of p53, promoting LSC survival. We hypothesize that enzymatic/non-enzymatic role of HDAC8 is critical for LSC survival but not for HSCs. Then, we characterized our two tetrahydroisoquinoline (TIQ)-based selective HDAC8 inhibitors (HDAC8i) BIP and OCH3 for growth inhibition, apoptosis, activation of caspase 3, integrity of mitochondrial membrane potential (MMP), and acetylation of histone H4 in human leukemia cell lines. The growth inhibitory effects observed in cell lines were validated using bone marrow (BM) or peripheral blood (PB) cells from AML patients. Colony forming cell (CFC) assays were performed using AML BM/PB cells treated with OCH3 or BIP. OCH3 and BIP were also tested for hematotoxicity using normal CB CD34+ cells. Furthermore, we compared class I HDAC isoform engagement in human normal cord blood (CB) CD34+ cells and in SET-2 leukemia cells using our novel photoreactive probe TH1143. In CD34+ cells, TH1143 had higher level of engagement for HDAC1 and 2, whereas engagement of HDAC3 and 8 was minimal. In SET-2 cells, HDAC3 and HDAC8 displayed relatively higher engagement with TH1143 indicating HDAC engagement is likely cell type specific.

Poster 44

Shreya Deb

Loss of intestinal SNX27 promotes barrier dysfunction, inflammatory responses, and tumorigenesis

BACKGROUND: SNX27 belongs to the sorting nexin (SNX) family of proteins that play a crucial role in the endocytic-recycling pathway and carry a conserved PX-domain. SNX27 is a unique member as it carries an additional PDZ-domain and mediates the recycling of endocytosed transmembrane proteins preventing their lysosomal degradation. Previously, SNX27 has shown to mediate neurodevelopmental processes and pathogenesis of Alzheimer's and Down's Syndrome. However, SNX27 is differentially expressed in other tissues and its role particularly in the intestinal physiology has not been explored yet. AIM: Intestinal tissue homeostasis and gut dysbiosis are regulated by several factors including the epithelial barrier. Tight-junction (TJ) and Adherens-junction (AJ) proteins maintain the epithelial barrier integrity while endogenously undergoing endocytosis and recycling. However, their interactions with SNX27 has not been validated yet. Here, we aim to determine the novel roles of SNX27 in regulating intestinal tissue homeostasis via epithelial barrier maintenance and its effects on the onset of gut inflammatory responses and tumorigenesis. METHODS: We have generated a novel mouse model of intestinal epithelial cell-specific deletion of SNX27 (SNX27_IEC). We analyzed basal level changes and challenged the mice with DSS-induced colitis and AOM/DSS-induced colorectal cancer (CRC). We also performed in vitro SNX27 loss-of-function studies via siRNA knockdown (KD) in SKCO15 cells. To determine the clinical relevance, we analyzed SNX27 expression in IBD and CRC patients using online datasets. RESULTS: SNX27_IEC mice weighed significantly less, had shorter colons, and heavier spleens compared to age-matched control mice. Deletion of SNX27 increased gut leakiness and induced apoptosis indicated by significantly upregulated cleaved Caspase-3 (pro-apoptotic) and downregulated Bcl-xL (anti-apoptotic) protein levels. TUNEL-staining also showed greater number of apoptotic cells in SNX27_IEC mice. The epithelial barrier integrity was disrupted in SNX27_IEC mice as TJ proteins, Claudin10 and ZO-1, as well as AJ proteins, _-catenin and E-cadherin, were significantly downregulated. SNX27_IEC mice had greater tissue infiltration of inflammatory cells and mRNA levels of pro-inflammatory cytokines IL-6 and TNF-_ were significantly higher. Consequently, we observed activation of NFkB pathway as SNX27_IEC mice had upregulated phospho-p65 protein expression. Upon challenging with intestinal disorders, SNX27_IEC mice were more sensitive towards DSS and AOM/DSS treatments as they lost more bodyweight, had leakier guts, shorter colons, and heavier spleens. SNX27_IEC mice exhibited early-onset of colonic tumors and had overall poor survival rate with colitis and CRC treatments. Meanwhile, inhibition of SNX27 in vitro reduced PCNA protein levels and delayed wound closure. However, SNX27-KD cells had higher nuclear translocation of phospho-p65 and rearranged distribution of downregulated _-catenin and E-cadherin, key mediators of epithelial-mesenchymal transition, indicating increased invasiveness. Lastly, human datasets GSE11223, 6731, 8671, 21510 revealed significant downregulation of SNX27 expression in IBD and CRC patients, suggesting clinical relevance of SNX27 in intestinal disorders. CONCLUSIONS: Overall, intestinal deletion of SNX27 promotes apoptosis, inflammation, and disrupts epithelial barrier that increases susceptibility towards colonic inflammation and tumorigenesis. Our results indicate a novel role of SNX27 in regulating intestinal tissue homeostasis. Therefore, understanding the mechanisms of SNX27 loss in IBD and CRC will provide insights into new prevention and therapeutic targets.

Poster 45

Tanushree Tanushree

MLK3-mediated phosphorylation of _-catenin facilitates cellular senescence and SASP-related Osteopontin secretion in HCC

Title: MLK3-mediated phosphorylation of _-catenin facilitates cellular senescence and SASP-related Osteopontin secretion in HCC Authors: Tanushree, Deepti Srivastava, Rong Ke, Ajay Rana, Basabi Rana* ABSTRACT: BACKGROUND: The canonical Wnt/_-catenin signaling pathway is an important mediator of normal liver growth and development. Abnormal activation of this signaling axis has also been linked with various liver pathologies, including hepatocellular carcinoma (HCC). In normal cells, _-catenin signaling is strictly regulated by a degradation complex that includes adenomatous polyposis coli (APC), Axin, Glycogen Synthase Kinase _ (GSK3_), Casein kinase 1_. Mutations of _-catenin itself and other genes regulating this pathway have been reported in HCC, leading to aberrant activation of this axis. AIM: In earlier studies, we have reported that Mixed Lineage Kinase 3 (MLK3), which is a member of MAPK kinase Kinase (MAP3K) family can phosphorylate and stabilize _-catenin and regulate its downstream pathway. The biological consequences of this phosphorylation, however, are unknown. The current studies were aimed to elucidate the detailed mechanism regulated by MLK3-_-catenin axis in HCC. METHODS: Towards fulfilling this knowledge gap, stable HCC cell lines overexpressing _-catenin were generated. RT2 Profiler PCR Array was done to identify novel _-catenin downstream target(s), and the results were validated by qPCR, Immunofluorescence, ELISA, IHC and _-Galactosidase (_-Gal) assays. RESULTS: Using Mass Spectrometry, here we identified that MLK3 can phosphorylate the N-terminal region of _-catenin at Serine 129 site. To understand the significances of _-catenin S129 phosphorylation, stable HCC cell lines overexpressing _-catenin wild-type (_-catenin WT), phospho-deficient mutant of Serine 129 (_-catenin-S129A) and the phospho-mimetic mutant of Serine 129 (_-catenin-S129D) were generated. Interestingly, our _-Gal assays revealed that overexpression of _-catenin WT and S129D promotes a senescence phenotype in the HCC cells, which was inhibited in the S129A mutant. These cells also showed increased expression of a set of unique senescent genes including COL1A1, COL3A1, IGFBP5, IGFBP7, and TGFB1I1, which were dependent on S129 phosphorylation. Analysis by RT2 Profiler PCR Array for cytokine and chemokine genes showed an increased expression of SPP1 (Osteopontin gene) in the WT compared to S129A cells. Further validation by qPCR, Immunofluorescence, and ELISA confirmed the induction and secretion of Osteopontin by _-catenin WT and S129D but not by S129A, suggesting a senescence-induced secretory phenotype (SASP) mediated by _-catenin-S129 phosphorylation. TMA analysis showed significant increase in the expression of Osteopontin in HCC patient samples compared to the normal samples. Database analysis suggested that Osteopontin gene is induced in HCC, and high expression of Osteopontin is associated with poor prognosis. In addition, there is a strong correlation between CTNNB1 (_-catenin gene) and SPP1 gene expressions in HCCs. CONCLUSIONS: Considering a well-established role of the multifunctional cytokine Osteopontin in HCC and other liver pathologies, these results suggest a potentially novel paracrine signaling network operating in liver cells mediated via a crosstalk of MLK3-_-catenin-Osteopontin axis. These also suggest MLK3 to be an ideal drug target for antagonizing this axis and towards successful clinical HCC management.

Poster 46

Serena C Thomas

Endothelial ACKR1 Facilitates the Formation of the Pre-Metastatic Niche in the Lung

Background: Metastasis is the leading cause of breast cancer-related mortality, however, the molecular mechanisms behind metastasis are still largely unknown. During metastasis, tumor cells detach from the primary tumor and spread to distant organs. From there, it is hypothesized that tumor cells mimic mechanisms used in leukocyte extravasation(LE) to extravasate through the endothelial cells of the vessel wall in a process known as tumor cell extravasation(TCE). Several genes mediate LE by priming the endothelial cells for extravasation. Particularly, Atypical Chemokine Receptor 1(ACKR1), a promiscuous chemokine receptor, has an established role in promoting LE via localization of chemokines to endothelial cell junctions. The role of endothelial ACKR1 as a potential actor in breast cancer cell extravasation has not been studied. Hypothesis: We hypothesize that endothelial ACKR1 is upregulated at distant metastatic sites, leading to increased breast cancer cell extravasation and ultimately increased metastatic spread and mortality. Methods: To understand ACKR1 function, we investigated the expression of endothelial ACKR1 at the distant metastatic site of the lung, a common site of breast cancer metastasis. We analyzed lung endothelial ACKR1 expression using immunofluorescent staining of lung vasculature over time during tumor progression in immunocompetent mice bearing orthotopically implanted E0771.LMB breast tumor cells. We then analyzed the timing of metastatic tumor arrival in the lung and localization of disseminated tumor cells near Ackr1-positive or Ackr1-negative venules. To study the impact of tumor-secreted factors on lung endothelial ACKR1 expression, we analyzed lung sections collected from day 3- and day 7- serum-free media (SFM) or ELCM (E0771.LMB conditioned media) treated mice._ Using immunofluorescent staining, we also analyzed these sections for innate immune cell infiltration. Results: Our results revealed a significant upregulation of endothelial ACKR1 in the lungs of TB mice. This increased ACKR1 expression was specifically localized to the pulmonary venule endothelium. Additionally, we observed that disseminated tumor cells were in closer proximity to ACKR1+ venules vs ACKR1- venules. Finally, tumor-conditioned media alone also significantly upregulated endothelial ACKR1 in the lungs at Day 7 of treatment compared to SFM and a significantly higher percentage of macrophages were present in the lungs of these mice. Conclusions: Our results have demonstrated how endothelial ACKR1 in the distant metastatic site of the lung is induced by breast tumor-secreted factors and that vessels expressing ACKR1 may promote breast cancer metastasis. The increased presence of disseminating tumor cells near ACKR1+ venules suggests that ACKR1 is a major player in tumor cell extravasation, leading to subsequent metastasis. For future experiments, we will further explore potential secreted factors responsible for Ackr1 upregulation in the lung.

Poster 47

Elizabeth N Kaweesa

Didesmethylrocaglamide Cytotoxic Activity in High Grade Serous Ovarian Cancer

BACKGROUND, PURPOSE, or OBJECTIVES: Ovarian cancer is the fifth leading cause of death in women with High Grade Serous Ovarian Cancer (HGSOC) as the deadliest form of ovarian cancer accounting for 70% of all cases. The American Cancer Society estimates that there will be 19,680 new diagnoses and 12,740 women will die from this cancer in 2024 alone. The majority of ovarian cancer patients present with late-stage disease that has spread throughout the abdomen due to lack of early screening and detection methods. Current treatment strategies include cytoreductive surgery and lifesaving chemotherapy. Patients eventually ultimately fail to respond to current therapies, including paclitaxel/Taxol and platinum, leading to relapse and eventually death. Therefore, new drug treatment and therapeutic strategies are needed for better prognosis. Natural products are an important source of drugs as they account for 50% of Federal Drug Administration (FDA) approved drugs used in the clinic today. These are secondary metabolites derived from natural sources like plants, microbes, and marine environments. HYPOTHESIS/AIMS: Our research seeks to study the efficacy and mechanism of the novel natural products didesmethylrocaglamide (DDR) and phyllanthusmin34 (PHY34) isolated from plant species. We hypothesize that these compounds effectively kill HGSOC with unique mode of action and may be used in combination with current therapies to increase targeted therapy in more patients. MATERIALS or METHODS: The effects of DDR and PHY34 were studied using ovarian cancer cell lines as well as mouse models. Cancer cell-based experiments, fluorescence imaging, molecular biology techniques like western blot and proteomics were used to determine the mode of action of these compounds in ovarian cancer. RESULTS: DDR displays nanomolar cytotoxic activity in high grade serous ovarian cancer (HGSOC) cell lines as early as 24 hours. In addition, DDR is cytotoxic in the PE04 and MCF7ADR cell lines that are resistant to current treatments cisplatin and paclitaxel, respectively. Increased cytotoxicity is observed with the combination treatment of DDR and PHY34 as well as reduced tumor burden in mice studies. CONCLUSIONS: The effective cytotoxicity of DDR and PHY34 in drug sensitive and resistant cell models suggests potential new therapeutic strategy against HGSOC to tackle chemoresistance.

Poster 48

Andrew P McLeod

Differences in Cognitive Performance Between Breast and Prostate Cancer Survivors

Background: Up to 75% of cancer survivors experience cognitive impairment from cancer or its treatment. This impairment can last up to 10 years. While many studies have focused on single cancers, very few have compared cognition between survivors of different types of cancer, and none have done so in a nationally representative sample. Furthermore, none have investigated the relationship of modifiable risk factors, such as diet, with cognitive impairment in cancer survivors and whether those relationships differ by cancer type. Results from this type of investigation may better inform which cancer patients may benefit most from a healthful diet. Aims: The aims of this research are to 1) determine if there are differences in cognitive performance between breast and prostate cancer survivors and 2) determine if these differences vary by Mediterranean Diet (Med Diet) adherence. Methods: We used data from the 1999-2002 and 2011-2014 National Health and Nutrition Examination Survey and adjusted linear regression to determine if prostate cancer survivors had different cognitive performance relative to breast cancer survivors. We then tested whether Med Diet was an effect modifier of the association using an interaction term (CancerType*Med Diet). Med Diet adherence was defined using the alternative Mediterranean Diet (aMed) score which gives 1 point for intake above the sample-specific median for food groups highly abundant in a Med Diet, such as fruits and vegetables, and 1 point for intake below the sample-specific median for food groups in low abundance in a Med Diet, such as red and processed meats (range 0-9). aMed score was dichotomized at the median (i.e., low versus high adherence). Cognitive performance was measured as the number correct on the Digit Symbol Substitution Test (DSST), which assesses processing speed, attention, and working memory. Models were adjusted for age, race, education, income, comorbidity burden, smoking status, total kilocalories, day of recall, time of year of recall, and testing environment. Results: The sample included 342 cancer survivors, 156 with breast and 186 with prostate cancer. Participants mean age was 72.7 years (SE=0.5). The average DSST score was 44.8 (SE=1.0) for prostate and 50.6 (SE=1.4) for breast cancer survivors. The average aMed score was 3.7 (SE=0.2) for prostate and 3.6 (SE=0.2) for breast cancer survivors. In adjusted models, prostate survivors, compared to breast cancer survivors, had significantly lower DSST scores (b = -6.4, 95% CI: -9.7, -3.1). This association varied by Med Diet (p for difference <0.05); the DSST score for prostate cancer survivors with high Med Diet adherence was not significantly different than breast cancer survivors (b = -3.2, 95% CI: -7.6, 1.3) but among those with low Med Diet adherence, the score was significantly lower among those who had prostate cancer (b = -10.3, 95% CI: -14.5, -6.0). Conclusions: Prostate cancer survivors had worse cognitive performance than breast cancer survivors. The association varied by Med Diet adherence whereby prostate cancer survivors had similar scores to breast cancer survivors if they were highly adherent to a Med Diet. Prospective studies should be conducted to confirm these results.

Poster 49

Adriana Duraki

Vitamin D deficiency leads to a proinflammatory microenvironment in the prostate that supports carcinogenesis

Background: Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men. However, its impact is pronounced among African American (AA) men, who face double the mortality rate compared to their European American (EA) counterparts. Melanin-related vitamin D (vitD) deficiency in AA men may contribute to the heightened risk of lethal and aggressive PCa. VitD is a hormone with a pivotal role in skeletal maintenance and bone health, however, it also has anti-tumor, immuno-modulatory, and pro-differentiation effects. The prostate microenvironment, comprising a complex network of cells and signaling molecules, plays a crucial role in regulating various physiological processes within the prostate. Prostate immune infiltrates play a pivotal role in maintaining homeostasis by secreting cytokines crucial for effective immune surveillance and defense against abnormal cell growth. Therefore, vitD deficiency may shift immune cell subpopulations in the prostate, fostering an inflamed environment. The prostate stroma actively influences the epithelial cells by secretion growth factors, chemokines, and cytokines. Here, we examined how vitD deficiency in male mice alters molecular profiles of stromal and immune cells within the prostate. Hypothesis/Aims: We hypothesize that vitD deficiency alters the prostate microenvironment to support carcinogenesis. Methods: Male C57Bl6 mice were fed either a vitD deficient or control diet for 6 months. Dorsolateral prostate lobe and cells were isolated for single-cell RNA sequencing. Gene expression differences and cell population analyses were conducted in "R". Results: Multiple types of immune cells and stroma were identified in the scRNAseq analyses. Numerous differentially expressed genes were identified within the stroma, with known roles in inflammation, paracrine signaling to the epithelium, and tumor microenvironment remodeling. Additionally, our analysis revealed a profound shift in immune cell infiltrates in the prostate. We observed increased expression of activated T cell markers in mice with vitD deficiency, which may contribute to an immunosuppressive environment. In contrast, mice on a sufficient diet expressed markers of central memory T cells, poised to respond against cancerous cells, suggesting differing cancer surveillance capabilities based on vitD status. Conclusions: These data suggest that maintaining adequate vitD levels may be essential for regulating the prostate microenvironment and preventing the molecular changes that may contribute to earlier and more aggressive PCa development. VitD deficiency status may lead to heightened inflammation and T cell activation. As vitD is easily supplemented, our studies offer a rationale for preventing vitD deficiency to support prostate health and function, potentially reducing the risk of aggressive PCa. Given the vulnerability of AA men to vitD deficiency and lethal PCa, this research aims to mitigate the disparity by addressing the underlying factors. As our findings highlight shifts in immune infiltrates within the prostate due to vitD deficiency, interventions to maintain optimal vitD levels could potentially improve treatment outcomes for AA patients facing PCa.

Poster 50

Rishi Patel

The Role of Endothelial ACKR1 in Breast Cancer Metastasis

Background: Metastasis is the leading cause of death in breast cancer patients, responsible for 75% of breast cancer-related deaths. A critical step in metastasis is tumor cell extravasation, where circulating tumor cells exit blood vessels to establish metastases in distant organs. Atypical Chemokine receptor 1 (ACKR1), a non-signaling chemokine receptor expressed on endothelial cells (EC) and red blood cells, has emerged as a potential regulator of breast cancer cell extravasation based on its ability to promote leukocyte extravasation. Hypothesis: We hypothesize that endothelial ACKR1 is upregulated at distant metastatic sites, resulting in increased breast cancer cell extravasation and, ultimately, increased metastatic spread and mortality. At the primary tumor, cancer cells secrete inflammatory factors into circulation. These secreted factors can then travel to distant organs, like the lung which is a common site of breast cancer metastasis, and cause the endothelial cells to upregulate ACKR1 expression. Once tumor cells eventually arrive at these distant organs, they will then be able to extravasate out of blood vessels more readily through the ACKR1+ endothelial cells. Methods: To investigate this hypothesis, we orthotopically implanted mouse breast cancer cells into the fourth mammary fat pad of wildtype and Ackr1 Null mice and measured for changes in primary tumor growth and lung metastases. Since we were interested in the specific role of endothelial Ackr1, we generated and validated an endothelial specific Ackr1 knockout mouse model (ACKR1 ECKO). Additionally, we conducted a time course experiment comparing endothelial Ackr1 expression in the lungs of tumor-bearing (TB) versus non-tumor-bearing (NTB) mice by performing immunofluorescent staining of the lungs throughout breast cancer progression. Results: Our results show, that global Ackr1 knockout results in significantly reduced lung metastases compared to control. We successfully validated endothelial-specific Ackr1 knockout in the ACKR1 ECKO mouse model, while preserving RBC Ackr1 expression. Additionally, endothelial Ackr1 expression significantly increased in TB mouse lungs starting at day 13 and continuing through day 28 . Conclusion/Future Directions: We have determine that pulmonary Ackr1 expression is induced by breast tumors and plays an important role in breast cancer metastasis progression. Investigating the factors responsible for endothelial ACKR1 upregulation during breast cancer progression promises new avenues for targeted treatments for patients at high-risk for metastatic disease.

Poster 51

Monica MJ. Chen

Conditional Axin1 Deletion Alters Host's Susceptibility to Colitis and Colitis-Associated Colorectal Cancer

BACKGROUND Axin1 is traditionally viewed as a key negative regulator of _-catenin within Wnt signaling pathway. It has been linked to multiple cancers including liver, stomach, and colon cancers. Emerging evidence suggests Axin1's function extends beyond Wnt pathway, possibly affecting other critical signaling pathways such as MAPK, PIK3, and p53, contributing to cell growth, apoptosis, inflammation, and tumorigenesis. In our prior investigation, we observed that deleting Axin1 in intestinal epithelial cells (Axin1ΔIEC) shielded mice from DSS-induced colitis model. Consequently, we investigated how Axin1 deletion in IECs cells influences tumorigenesis using a colitis-associated colorectal cancer model. Because Axin1's role in immune-related pathways lacks clear mechanistic understanding, we employed a mouse strain with Axin1 knockout in myeloid cells (Axin1ΔLYZ) to address this knowledge gap. HYPOTHESIS This study aims to examine Axin1's role in gut homeostasis and the progression of colitis and colorectal cancer, employing cell specific conditional Axin1 knockout mouse models. We propose that Axin1 is pivotal in shaping the gut microbiome and regulating the susceptibility to colitis and colorectal cancer. Furthermore, we hypothesize that Axin1 affects the immune system and loss of Axin1 in myeloid cells might compromise their essential functions. MATERIALS Axin1LoxP mice were bred with villin-cre, Defa6-cre, or lyz-cre mice to specifically delete Axin1 in IECs, Paneth cells (PC), and myeloid cells (LYZ), respectively. Fecal samples were collected for the 16s rRNA and metagenomic analysis for the microbiome profile and function in these mice with Axin1 conditional deletion. In the colitis model, mice received 2% DSS in drinking water for 7 days and were euthanized on day 8, with colitis severity assessed through changes in body weight and the disease activity index. For the AOM/DSS-induced colitis-associated colorectal cancer model, mice were administered a single intraperitoneal (IP) dose of AOM, followed by three cycles of 2% DSS in drinking water for 7 days with 3 weeks of recovery between cycles, and were euthanized at week 11. Cancer severity was evaluated based on the number, size, and location of colorectal tumors. RESULTS: At the basal line without any treatment, mice with Axin1 conditional showed altered microbiome. 16S rRNA analysis revealed that Axin1ΔIEC mice had an increase in the genus Ruminiclostridium6. Interestingly, Axin1ΔLyz mice had depletions in the genera Anaeroplasma and Erysipelatoclostridium and enrichments in Alloprevotella.KEGG analysis indicated that DNA replication and repair was significantly increased in the Axin1ΔLyz mice, compared to the Axin1LoxP control mice. Axin1ΔLYZ mice exhibited an increased sensitivity to DSS, evidenced by a higher mortality rate upon 2% DSS treatment. In the AOM/DSS model, Axin1ΔIEC exhibited an 88.9% survival rate, Axin1LoxP 54%, Axin1ΔPC 37.5%, while Axin1ΔLYZ had the lowest at 13.6%. Notably, Axin1ΔLYZ mice demonstrated a significant increase in tumor numbers compared to Axin1LoxP, Axin1ΔIEC, and Axin1ΔPC mice. CONCLUSIONS Removing Axin1 from myeloid cells heightens susceptibility to AOM/DSS-induced tumorigenesis. These findings indicate Axin1 plays a complex tissue specific role in intestinal and microbial homeostasis, potentially affecting innate immunity. Further studies of Axin1 dysfunction in colon cancer are needed to elucidate the exact mechanism in which Axin1 regulates tumorigenesis for better prevention and treatment.

Poster 52

Rose Bahari

"Targeting Melanoma Cells with G-Quadruplex-Forming Oligonucleotide T-Oligo: Enhanced Efficacy through Combination Therapy"

BACKGROUND, PURPOSE, or OBJECTIVES: Melanoma is one of the deadliest skin cancers in the world, estimated to cause 7,990 deaths annually. Telomerase binds to the 3' overhang of the telomere, causing telomere elongation and cell survival. Treatment against melanoma aims at targeting the 3' telomere overhang using T-oligo which is a guanine-rich oligonucleotide homologous to the telomere overhang, preventing the growth of melanoma cells by inducing apoptosis. As a guanine-rich oligonucleotide, T-oligo can form G-quadruplexes (G4), secondary structures of nucleic acids, which we aim to detect using FRET microscopy in melanoma cells. Currently, vemurafenib and trametinib are agents that are used to treat melanoma. We were also interested in studying the combinational treatment of T-oligo with vemurafenib/trametinib to determine if it improves the efficacy of these drugs as compared to monotherapy in melanoma cells. HYPOTHESIS/AIMS: We hypothesize that T-oligo forms G-quadruplexes in the nucleus of melanoma cells and combination therapy of vemurafenib/Trametinib with T-oligo would be efficacious compared to monotherapy. MATERIALS or METHODS: The role of T-oligo was evaluated in melanoma cancer cell lines. MM-AN cells were plated in triplicates in 6-well plates, and they were given single-labeled and double-labeled T-oligo of length 11 mer and 22 mer with FITC on the 5'-end and Cy3 on the 3'-end. The uptake was evaluated with fluorescence microscopy and formation of G4 which were evaluated with FRET microscopy acceptor photobleaching. MTT assay was used to test the efficacy of T-oligo, vemurafenib, or trametinib in two cell lines, WM35 and 451Lu. Two thousand cells/well were plated in a 96-well plate and allowed to adhere for 48 hours before being treated with diluent (media), T-oligo alone, vemurafenib or trametinib alone, or a combination of T-oligo with vemurafenib or trametinib. After 96 hours of treatment drug, an MTT assay was performed. RESULTS: After incubating the melanoma cells with T-oligo, approximately 90% of T-oligo was found to localize in the nucleus of melanoma cells. The donor fluorophore was FITC, and the acceptor fluorophore was CY3. For FRET detection, we used the acceptor photobleaching method in which the acceptor (Cy3) was completely photobleached, and any corresponding increase in the donor's intensity was detected and quantified. We found a significant increase of 40% - 150% in donor intensity after acceptor photobleaching in multiple melanoma cell lines. This may be due to G-quadruplex formation causing FRET, thus indicating that T-oligo may be utilizing this mechanism of action for its anticancer effects. The IC50 values for different cell lines were obtained from the MTT analysis and showed that T-oligo with either Vemurafenib or Trametinib had an additive or more additive effect in all cell lines tested. CONCLUSIONS: Acceptor photobleaching results from the FRET microscopy showed increased intensity of melanoma cells, thus confirming the formation of a G-quadruplex in T-oligo. Additionally, the lower IC50 values when T-oligo was used in combination therapy confirmed the hypothesis that combinational therapy of T-oligo is more effective than monotherapy.

Poster 53

Katherine A. Alexander

Systemic Notch4 inhibition and endothelial-specific Notch4 ablation regulate breast cancer growth in distinct and opposing manners.

BACKGROUND: Despite advances in screening and care, breast cancer is the most highly diagnosed cancer and the second highest cause of cancer fatality in women. Notch1 signaling regulates blood vessel growth and is a critical regulator of tumor angiogenesis. Small molecule and antibody treatments that block Notch signaling or target the Notch1-Dll4 signaling axis have shown anti-tumor efficacy, but gastrointestinal toxicity has limited clinical applications. A second Notch family member, Notch4, is relatively poorly studied and has not previously been targeted clinically. In recent work, our lab demonstrated that treatment with a first-in-class anti-Notch4 antibody, E7011, substantially inhibited tumor growth and vascular function, and promoted dramatic increases in tumor macrophages and a shift toward anti-tumor macrophage phenotypes. Sequencing and immunohistochemical analysis of tumors suggested that Notch4 expression was restricted to endothelial cells in responsive tumor types, suggesting that endothelial Notch4 promotes tumor growth via endothelial-immune interactions. However, we have recently developed an endothelial-specific Notch4 knockout (Notch4ECKO) and determined that specific ablation of endothelial Notch4 increases tumor growth in multiple models of breast cancer. HYPOTHESIS: The opposing effects of systemic Notch4 inhibition and endothelial-specific Notch4 loss on tumor growth leads us to hypothesize that endothelial Notch4 regulates breast cancer in a manner distinct from other sources of Notch4 in the body. METHODS: We have implanted syngeneic mouse mammary tumor cells orthotopically into either the fourth or fifth mammary fat pad of immunocompetent C57BL6 mice. We have used E0771.LMB and PY8119 cells to model luminal and triple negative breast cancer, respectively. To systemically inhibit Notch4, we have treated mice with 25 mg/kg E7011 or isotype human IgG controls. To specifically remove endothelial Notch4, we have developed a Cre-mediated tamoxifen inducible endothelial-specific knockout (Notch4ECKO) mouse model. We have examined tumor growth and vascular coverage. RESULTS: Treatment with anti-Notch4 E7011 antibody led to reduced breast PY8119 tumor growth compared to human IgG control treatment when tumor cells were implanted into either the 4th or 5th fat pad. E7011 treatment increased tumor vessel area and perfusion, increased macrophage recruitment, and decreased fibroblast activation. By contrast, PY8119 tumors implanted into the 4th or 5th fat pad of Notch4ECKO mice were significantly larger compared to Notch4WT controls. We confirmed this with a second mammary carcinoma cell line, E0771.LMB. CONCLUSIONS: Inhibition of endothelial Notch4 with a novel neutralizing antibody (E7011) decreases tumor growth, increases macrophage recruitment, and decreases fibroblast activation in mouse models of breast cancer. In mice with Notch4 genetically deleted from the endothelium lining the tumor vasculature, tumors have been shown to be significantly larger and thus points to a distinct role for endothelial Notch4 from the effects of anti-Notch4 E7011 treatment.

Poster 54

Anthony X. Rodriguez

Comparing Potential Tumorigenicity and Toxicity of Clean Energy HQ-115 and Legacy PFOS

Background Poly- and perfluoroalkylated substances (PFAS) are chemicals found in many materials that resist heat and repel stains, grease, and water. Some studies have shown PFAS to be associated with several negative health outcomes. Some health concerns include cardiovascular dysfunction, increased cancer risk, and high cholesterol. New PFAS are produced yearly, such as lithium bis-trifluoromethanesulfonimide (HQ-115). Literature regarding PFAS is plentiful regarding toxicity and tumorigenesis; however, there is a significant lack of information on this substance. Methods In this study, HQ-115 was exposed to xenografted nude mice daily for 15 days in various concentrations to examine the potential toxicity and tumorigenesis that may result from constant exposure. The mice had their xenografts, testes, kidneys, livers, and blood harvested for analysis after the study had concluded. The evaluation included organ weight, tumor volume, and cell viability analysis. Results Liver weight resulted in a statistically significant increase when exposed to PFOS. However, when exposed to HQ-115 in both concentrations, there was no significant change. HQ-115 carries the same tumorigenic traits as previous PFOS, indicating its ability to increase tumor volume. Cell viability assays showed when exposed to HQ-115, both cell lines expressed higher cell viability at the 250 QM concentration in all trials. Conclusions HQ-115 may be slightly less toxic than previous PFOS; however, it carries the same tumorigenic traits as previous PFOS. Based on this initial analysis HQ-115 is just as tumorigenic as previous PFOS and should be looked into for regulation. Further analysis is required.