



**15th Annual Spotlight on Early Career Investigators—
A Cancer Research Mini-Symposium**

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ABSTRACTS

Oral Presentation Abstracts

LYNCH SYNDROME: THE KNOWN AND UNKNOWN CONSEQUENCES OF MSH2 AND MSH6 DEFICIENCY

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Abstract: Lynch syndrome is a hereditary disorder that predisposes patients to cancer. Lynch syndrome patients have germline mutations in the genes encoding the mismatch repair proteins, including MSH2 and MSH6. In addition to their role in mismatch repair, MSH2 and MSH6 also function in the regulation of the homologous recombination (HR) DNA repair pathway. This additional function of MSH2 and MSH6 suggests that loss of regulation of HR may also drive tumorigenesis in MSH2- and MSH6-deficient patient tumors. HR is the primary high-fidelity mechanism of DNA repair in the cell. In vivo studies indicate that MSH2 and MSH6 act to prevent recombination between mismatched, or “homeologous”, regions. This is significant, because homeologous recombination can cause mutagenesis and genome rearrangements. However, the mechanism through which MSH2 and MSH6 achieve this outcome is currently poorly defined. Moreover, the clinical significance of loss of this regulation in MSH2- and MSH6-deficient patient tumors is unknown due to limitations in the DNA sequencing technologies applied.

This project aims to address these gaps in the published literature by using combination of genetic, genomic, and biochemical methodologies. To map the factors responsible for preventing homeologous recombination in vivo in budding yeast, I will adapt two proximity ligation-based assays recently developed by the Heyer laboratory (1-3) to detect recombination intermediates formed between the site of a DNA double-stranded break and matched or mismatched donors. I will then reconstitute this mechanism using purified budding yeast and human proteins. In addition, I will use long-read sequencing to sequence matched tumor and blood samples from patients with MSH2- and MSH6-deficient tumors. Long-read sequencing has the ability to identify genome rearrangements caused by promiscuous recombination between mismatched, repetitive elements, whereas short-read sequencing cannot identify repeat recombination between regions longer than the length of the sequenced fragment. Sequencing analysis of patient tumors will allow us to determine the contribution of loss of regulation of HR by MSH2 and MSH6 to tumorigenesis. Eventually, this work has the potential to lead to improved diagnostic testing and targeted therapies to treat these tumors.

This project was recently initiated, and I will present the concept and preliminary results.

Citations

1. Piazza A, et al. (2019) Dynamic Processing of Displacement Loops during Recombinational DNA Repair. *Mol. Cell* 73(6):1255-1266.
2. Piazza A, Koszul R, & Heyer WD (2018) A Proximity Ligation-Based Method for Quantitative Measurement of D-Loop Extension in *S. cerevisiae*. *Mechanisms of DNA Recombination and Genome Rearrangements: Intersection between Homologous Recombination, DNA Replication and DNA Repair*, *Methods in Enzymology*, eds Spies M & Malkova A), Vol 601, pp 27-44.
3. Piazza A, Koszul R, & Heyer W-D (2018) A Proximity Ligation-based Method for Quantitative Measurement of D-loop Extension in *S. cerevisiae*. *Methods Enzymol.* 601:27-44.

RESISTANCE TO OLAPARIB IS DEPENDENT ON RE-EMERGENCE FROM G2/M ARRESTED SENESENCE

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Background: Inhibition of poly (ADP-ribose) polymerase (PARP) is an exciting treatment strategy recently approved for prostate cancer patients with homologous recombination repair defects. Despite this advance in the field, there are important unanswered questions regarding PARP inhibitor (PARPi) use; 1) How do PARPi sensitive cells respond to treatment? 2) What mechanisms give rise to PARPi resistance? To address these questions, we sought to characterize response to PARP inhibition using PARPi sensitive LNCaP and C4-2B cells and two PARPi resistant cell line derivatives.

Methods: LN-OlapR and 2B-OlapR olaparib resistant cell lines were generated from LNCaP and C4-2B cells through chronic exposure to increasing doses of olaparib. Western blot was used to detect PARP activity, apoptosis, and DNA damage. Flow cytometry and beta-galactosidase activity assays tested response to PARPi's. CDK1 was inhibited using RNAi and small molecule drug, BMS-265246.

Results: OlapR cells exhibit marked resistance to olaparib versus parental cells. OlapR models are also cross-resistant to other clinically relevant PARPi's including rucaparib, niraparib, and talazoparib. Mechanistically, PARPi treatment inhibits PARP catalytic activity, induces DNA double strand breaks, and activates apoptosis in LNCaP and C4-2B cells. We also observed a cytostatic response in a significant proportion of cells. Flow cytometry showed a robust G2/M arrest in response to olaparib treatment, accompanied by marked increases in p21 expression and beta-galactosidase activity, suggestive of senescence. In contrast, OlapR cells do not exhibit G2/M arrest, increased p21, or senescence in response to PARP inhibition, suggesting that resistance is dependent upon re-emergence from p21 dependent senescence. CDK1 activity governs the G2/M cell cycle phases and is a primary p21 target. Thus, we tested if CDK1 inhibition re-sensitizes OlapR cells to PARPi treatment. Indeed, we found that CDK1 inhibition by either siRNA or BMS-265246 re-sensitized OlapR cells to treatment.

Conclusions: We find that response to PARP inhibition is characterized largely by a G2/M arrested senescence, which may give rise to resistance through re-emergence from this state. PARPi induced senescence provides an escape route from PARPi cytotoxicity, creating a repository of persistent cells which can give rise to resistance. Targeting CDK1 may prove to be an efficacious strategy for the treatment of re-emerged, PARPi resistant prostate cancer.

INTRAOPERATIVE FLUORESCENCE LIFETIME IMAGING (FLIM) FOR TUMOR DELINEATION AND SURGICAL GUIDANCE OF ORAL CAVITY AND OROPHARYNGEAL CANCER

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Background: Head and neck (H&N) squamous cell carcinoma (SCC) of unknown primary is a rare and challenging tumor cohort which represents approximately 2% of all new cases of H&N SCC. These tumors evade detection after exhaustive clinical, radiographic, and surgical evaluation. Ultimately, most primaries are detected or emerge as oropharyngeal cancers, particularly those that are p16+.

Given the importance of locating the primary cancer for providing optimal care, we evaluated the potential of a novel Fluorescence Lifetime Imaging (FLIm) technique for intraoperatively detecting these tumors.

Methods: A FLIm apparatus (UV laser excitation at 355 nm, with spectral autofluorescence emission evaluated at 390/20 nm, 470/14 nm, 542/25 nm, and 629/26.5 nm) was integrated into the da Vinci SP transoral robotic surgical platform. This platform was utilized to investigate the diagnostic potential of FLIm-derived parameters (average lifetime values and spectral ratios) in evaluating p16+ unknown primary carcinomas of the oropharynx in human patients (N=7). For each patient, time and spectrally resolved fluorescence information was acquired using a custom-built fiber optic probe which was used to bilaterally scan each patient's palatine tonsil, glossotonsillar sulcus region, and base of tongue. These FLIm measurements were combined with white-light endoscopy videos to generate views of the surgical region augmented with FLIm parameter maps. En bloc mucosal resection of the scanned tissues was then performed to identify occult malignancy. Histopathology was used to register the histopathological status of the excised tissue to the fluorescence lifetime data.

Results: Among the 7 patients, SCC of unknown primary was successfully located and excised in 3 patients (all p16+), comprising 1 palatine tonsil and 2 base of tongue tissues. No oropharyngeal primary was identified in final pathology for the other 4 patients. Receiver operating characteristic area-under-the-curve (ROC-AUC) was used to quantify the coregistered histologic true positive rate vs. false positive rate of the data on individual spectroscopic point-measurements output from a machine learning classifier. The classifier was trained on a larger 55 patient cohort of known primary oropharyngeal tumors, comprising 38 palatine tonsil and 17 base of tongue patients. Collectively, H&N SCC of unknown primary was detected with an ROC-AUC of 0.90 ± 0.06 indicating excellent discrimination. The mean sensitivity of the method was found to be $95 \pm 3.5\%$, and specificity $90 \pm 11.4\%$.

Conclusion: Our preliminary results demonstrate that for mucosa-presenting ($<250 \mu\text{m}$ from tissue surface) p16+ SCC of unknown primary, FLIm holds great promise in aiding a surgeon to screen, intraoperatively delineate, and excise these elusive tumor cohorts.

DEVELOPING A PRE-CLINICAL MODEL OF METASTATIC SOFT-TISSUE SARCOMA

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In 2021, an estimated 13,000 people will be diagnosed with soft-tissue sarcoma. While 65% of patients will survive, 35% will develop metastasis within 5 years and succumb to this disease. If the cancer has metastasized at the time of diagnosis, then only 15% of these patients will survive. This is a rare cancer that arises from mesenchymal stem cells and is comprised of over 50 subtypes, each histologically and genetically different. These include Undifferentiated Pleomorphic Sarcoma, Liposarcoma and Leiomyosarcoma. Despite their heterogeneity, each subtype can metastasize to the lungs, but the mechanism of metastasis is unknown. The rarity and complexity of this cancer makes it difficult to treat, and few models are available to study. As such, our aim for this project is to create models of metastatic sarcoma to compare the different sarcoma subtypes and identify mechanisms of metastasis. To accomplish this, we first injected human tumor cells into the calves of mice. Once grown, we collected and digested the primary tumor and metastasized lungs. To enrich for human cells in the lungs, we used magnetic beads to deplete mouse cells and then sorted for human cells in each sample. These crucial steps ensure we solely analyze human cells during downstream analyses. Following completion of the model, these enriched cells will be used for RNA, ATAC and Whole Exome sequencing. Through this we can characterize the different subtypes of sarcoma and compare the genetic profiles of primary and metastatic cells. This can identify future targets to prevent lung

metastasis in patients. Overall, the development of this model will advance our understanding of metastatic soft-tissue sarcoma and lead to improved therapies for patients.

NATURAL KILLER AND CYTOTOXIC T CELL IMMUNE INFILTRATES ARE ASSOCIATED WITH SUPERIOR OUTCOMES IN SOFT TISSUE SARCOMAS

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Background: Tumor infiltrating lymphocytes (TILs) have been shown to predict survival in soft tissue sarcomas (STS); however, the contribution of specific lymphocyte subsets such as natural killer (NK) and memory T cells to STS outcomes is undefined. We sought to characterize the extent of NK and memory T cell infiltration in STS to determine the correlation of these cytotoxic immune cells to outcomes.

Methods: Archived tumor tissue from 29 STS patients collected from 2008-2014 was evaluated. Tissue microarrays (TMAs) were constructed, and immunohistochemical analyses were performed by an STS pathologist for CD3, CD8, CD45RO, NKp46, TIGIT, and MHC-I. TIL scores of H&E slides were calculated. Metastasis-free survival (MFS) and overall survival (OS) were analyzed by Kaplan-Meier method.

Results: Among our cohort (mean age 56, 59% female), mean tumor size was 15.3 cm, consistent with a high-risk population. Majority of tumors (65%) were located on the extremity, 28% were retroperitoneal, and 7% trunk. The most common histologies were liposarcoma (34%), myxofibrosarcoma (21%), and pleomorphic sarcoma (21%) with 79% high grade. With a median follow up of 50 months, MFS and OS were 22 and 87 months, respectively. We confirmed a positive correlation between CD8+ T cell infiltration and significantly improved MFS ($P<0.05$), but not OS. Overall, NK cell infiltrates were low (median H score 0, range 0-66.5). However, we observed a trend for improved OS among patients with higher NKp46 scores (OS 68 months for NKp46 scores below median versus not reached for scores above median, $P=0.08$). The expression of NK inhibitory markers TIGIT and MHC-I correlated with T cell infiltration ($P<0.05$), but not NK cell infiltration.

Conclusion: Infiltration of cytotoxic lymphocyte subsets, including NK cells, is associated with superior OS in STS patients undergoing surgery. Further characterization of the immune infiltrate in STS may yield better biomarkers of prognosis and immune targeting.

MTAP DEFICIENCY RESHAPES THE IMMUNE MICROENVIRONMENT AND CONTRIBUTES TO TUMOR EVASION

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Methylthioadenosine phosphorylase (MTAP) deficiency occurs in a broad range of malignancies; notably, the loss of this purine- and methionine-metabolizing enzyme was found to be associated with poor survival in cancer patients. However, the mechanisms underlying tumor progression due to MTAP loss are yet to be elucidated. Utilizing integrated transcriptome analysis by RNA-sequencing and functional proteomics by reverse phase protein arrays, we demonstrated that MTAP deficiency

alters tumor-intrinsic, immune-related pathways such as CXCR4, IL-5, and IFN- γ signaling in both lung and kidney cancer cells. Data from Luminex assays confirmed that multiple cytokines with anti-tumoral properties including GM-CSF, IL-1 α , IL-1 β , IL-12 and IFN- γ are more abundant in cells with MTAP expression compared to MTAP-knockout cells. In addition to cytokine reprogramming, MTAP-knockout cells exhibited a marked increase in the immune checkpoint protein PD-L1. Upon co-culturing T cells isolated from human peripheral blood mononuclear cells with cancer cells, we found that MTAP loss-mediated PD-L1 upregulation inhibits T cell-mediated killing activity and induces several T cell exhaustion markers. In two xenograft tumor models, we showed that the average volume of tumors derived from MTAP-deficient cells is slightly greater than that of MTAP-proficient tumors. Surprisingly, a remarkable increase in tumor size was also observed in humanized mice bearing MTAP-deficient tumors, as compared to their MTAP-expressing counterparts. Our data suggest that MTAP loss downregulates the immune response and reprograms cytokines toward a tumor-favorable environment, promoting tumor growth and immune evasion.

SITE-SPECIFIC METABOLIC EFFECTS OF ACUTE NAPHTHALENE EXPOSURE IN MICE REVEALED BY METABOLOMICS OF MICRODISSECTED LUNG TISSUE

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Naphthalene is a ubiquitous environmental contaminant produced by combustion of fossil fuels and is a primary constituent of both mainstream and side stream tobacco smoke. Naphthalene elicits region-specific toxicity in airway club cells through CYP450-mediated bioactivation, resulting in depletion of glutathione and subsequent cytotoxicity. In vivo studies of naphthalene exposure have demonstrated increased formation of neoplasms following chronic inhalation, prompting the classification of naphthalene as a potential human carcinogen. While effects of naphthalene in animal models have been extensively studied, few experiments have characterized global changes in lung metabolic pathways. Specifically, responses to naphthalene exposure omitted analyses of individual lung regions. We here report on metabolomic changes in microdissected mouse lung airways and parenchyma obtained from animals sacrificed 2, 6, and 24 hours post-injection of naphthalene. Data on 577 unique identified metabolites were acquired by accurate mass spectrometry-based assays focusing on lipidomics and non-targeted metabolomics of hydrophilic compounds. Statistical analyses revealed distinct metabolite profiles between both major lung regions. In addition, the magnitude and number of statistically significant metabolites were different between lung airways and parenchyma for unsaturated lysophosphatidylcholines (LPCs), dipeptides, pyrimidines, and amino acids. Importantly, temporal changes were found to be highly distinct for male and female mice, with males predominantly exhibiting treatment-specific changes only at two hours post-exposure. In females, metabolic changes persisted until six hours post-naphthalene treatment, which may contribute to the previously characterized higher susceptibility of female mice to naphthalene toxicity. The present study highlights treatment-specific changes corresponding to lung remodeling, oxidative stress response, and DNA damage that may provide insights into potential mechanisms contributing to the previously reported effects of naphthalene exposure in the lung.

Poster Session Abstracts

NOVEL AUTOPHAGY INHIBITOR FOR THE TREATMENT OF PANCREATIC CANCER

M. Ramachandran, Z. Ma, Y. Li

In this study, we develop a novel autophagy inhibitor targeting one of the most aggressive cell populations present in pancreatic tumors, the pancreatic cancer stem cell (CSC). Targeting this population which has the malignant properties of tumorigenesis/tumor recurrence, the ability to differentiate into a heterogeneous tumor cell types and can self-renew is a new strategy to alleviate the current dismal 10% five-year survival rate of pancreatic cancer. Here we characterize an autophagy inhibitor which is a first-in-class nanoparticle comprised of a chloroquine-derived small molecule drug that can self-assemble into a micelle. Due to its favorable pharmacokinetic properties and the CSC reliance on autophagy, our compound is able to selectively target this cell population both in vitro and in vivo. Additionally, the compound has shown synergy with the current standard of care chemotherapeutic, gemcitabine, with an 88.9% reduction in tumor volume in the combination of the two drugs compared to gemcitabine treated alone ($p < 0.0001$) in two separate treatment regimens using a pancreatic PDX mouse model. We further illustrate that the stem cell population is targeted by inhibiting tumorsphere formation in established pancreatic cancer cell lines MIA-PaCa2 and PANC-1 and in pancreatic PDX-derived CSCs in vitro. The drug treatment also promotes a decrease in pluripotency markers, particularly in Sox2 levels. Finally, the drug is well-tolerated with a maximum tolerated dose above 100 mg/kg in mice and is orally bioavailable.

Breakout Room: 1 – Mythili Ramachandran

EVALUATING NUMBER OF PATHOLOGIC LYMPH NODES IN ORAL CAVITY CANCER

Samya Faiq, Roberto N Solis, Harveen Kaur Sekhon, Marianne Abouyared, Arnaud F Bewley, D. Greg Farwell, Andrew C Birkeland

Introduction: Currently, the 8th edition AJCC staging guidelines for HPV-associated oropharyngeal head and neck squamous cell carcinoma (HNSCC) has an entirely new staging paradigm based on pathologic nodal classification, while HPV-negative HNSCC nodal staging has been largely unchanged from the 7th edition. This study aims to evaluate the 8th edition AJCC pathological nodal staging system in oral cavity HNSCC.

Methods: A single institution retrospective case series study was performed that included patients diagnosed with oral cavity HNSCC who underwent resection with concurrent neck dissection between 2004-2020. The primary outcomes were five-year overall survival (OS), disease specific survival (DSS), and disease-free survival (DFS) to evaluate pathologic nodes using the 8th edition AJCC nodal staging used for HPV-associated HNSCC. Multivariate analysis was performed to adjust for disease characteristics and patient demographics and characteristics.

Results: Of 152 patients identified, 83 has 0 positive nodes, 56 had 1-4 positive nodes, and 13 had more than 4 positive nodes. Using 8th edition AJCC pathologic nodal staging system showed a difference between the groups for DSS ($p = 0.047$) and DFS ($p = 0.005$) but not for OS ($p = 0.053$).

Conclusion: Applying the 8th edition AJCC pathologic nodal staging system for HPV-associated oropharyngeal HNSCC to oral cavity HNSCC can be valuable for prognostication.

Breakout Room: 2 - Samya Faiq

BEREAVEMENT PRACTICES AMONGST HEAD AND NECK ONCOLOGIC SURGEONS

Harveen K Sekhon, Roberto N Solis, Samya Faiq, Marianne Abouyared, Arnaud F Bewley, D. Greg Farwell, Andrew C Birkeland

Introduction: HN squamous cell carcinoma is the 6th leading cancer worldwide by incidence with a 5-year overall survival of 40-50%. Coping with death is therefore inevitable in the career of a HN surgeon; however, the process of bereavement is sparsely discussed or formally taught. No studies to date explore the practice of bereavement among HN surgeons. This study aims to elucidate common bereavement practices and perceived barriers of practicing bereavement.

Methods: A 20-item anonymous questionnaire was formulated by the HN oncology team and a palliative care physician at the author's institution. Bereavement practices most commonly assessed in the literature were reconciled. A final version was approved by the American Head and Neck Society (AHNS) review committee and disseminated online through REDCap to all active AHNS surgeons between June 22, 2020 to July 6, 2020. Bereavement activity questions were combined and assigned values of 0-4 corresponding from "never" to "always" for multiple linear regression analysis. An attachment score was calculated based on questions related to the physicians' attachment to their patients, and these were assigned values 0-4.

Results: A total of 156 of 827 active AHNS surgeons completed the questionnaire (18.9%). Most respondents were male (75.0%), 37.2% had greater than 20 years of experience, 72.4% worked in academic centers, and 61.2% had practices mostly seeing HN cancer patients. Average bereavement activity score was low at 1.47 ± 0.66 , and the average attachment score was 2.08 ± 0.7 . A higher attachment score ($p < 0.001$) was correlated to increased bereavement activity. Gender ($p = 0.11$), experience ($p = 0.198$), practice environment ($p = 0.41$), and percentage of HN cancer patients seen ($p = 0.78$) were not predictors of bereavement activity. The most cited barriers to bereavement practices included being unaware of a patient's death (67.3%), needing to maintain boundaries (61.6%), lacking time (54.5%), and lacking training in this area (51.3%).

Conclusion: In general, HN oncologic surgeons do not commonly engage in bereavement practices, with many barriers cited. Among those who do, telephone calls or condolence letters to family are the most common bereavement practices. Feeling an attachment to patients, as determined by attachment score in this study, is the only predictor of increased bereavement activity.

Breakout Room: 3 - Harveen Sekhon

CHROMATIN ARCHITECTURE ORGANIZER BORIS MEDIATES ROR- γ REPROGRAMMING OF CHROMATIN LANDSCAPE IN ADVANCED PROSTATE CANCER

Yatian Yang, Junjian Wang, Hongye Zou, Christopher P. Evans, Hong-Wu Chen

Aberrations in 3D chromatin organization (e.g. topologically associating domains (TADs) and loops) have been implicated in cancer. Our previous study found that retinoid acid receptor-related orphan receptor γ (ROR- γ), a member of the nuclear receptor (NR) family of transcription factors, functions as a key determinant of androgen receptor (AR) overexpression and aberrant signaling in human castration-resistant prostate cancer (CRPC). However, the mechanism of ROR- γ control of AR function and chromatin landscape in CRPC is poorly understood. Our ChIP-seq and ATAC-seq-based epigenetic analyses revealed that treatment of CRPC cells with ROR- γ antagonists not only strongly suppressed genome-wide histone acetylation and H3K4 methylation but also markedly decreased the chromatin accessibility at enhancer and promoter regions. Interestingly, we found that the diminished chromatin accessibilities were largely overlapped with AR binding sites and gene activation-linked H3K27ac epigenetic marks. Our further analysis revealed that the ROR- γ antagonist not only diminished chromatin accessibility but also potentially induced "open" chromatin structures at gene regulatory regions, highlighting its remarkable activity in reprogramming chromatin structures. Importantly, we found that DNA binding motifs of a 3D chromatin regulator Brother Of the Regulator

of Imprinted Sites (BORIS) is highly enriched in chromatin regions with the reduced accessibility. Remarkably, knockdown of BORIS markedly downregulated the expression of ROR- γ and AR and inhibited the growth of CRPC cells. Therefore, our work reveals BORIS-ROR γ -AR as a novel regulatory axis in CRPC and provides new insights of the important role of aberrant chromatin architecture in advanced prostate cancer.

Breakout Room: 4 - Yatian Yang

DECIPHERING FORCE-DEPENDENT INTERACTIONS OF KERATIN AND CTEN USING IN VITRO MICRONEEDLE STRETCH

Gabriella Tzuwei Lai, Yuh-Ru Julie Lee, Volkmar Heinrich, Su Hao Lo, Soichiro Yamada
Epithelial tissues serve as an important protective structure and account for > 80 percent of all human cancer cases. Our current project focuses on keratin 8 (k8) and keratin 18 (k18) since these proteins are the primary intermediate filament proteins in simple epithelia. The keratin network provides mechanical integrity of epithelial tissues and may also serve as the force-sensing element in normal and cancer cells. Our lab has shown that cten, a protein known to act as both tumor suppressor and promoter, accumulates around force-bearing keratin fibers in vivo. Yet, the precise molecular interaction between keratin and cten remains ambiguous. To understand the mechano-biology of keratin and its implication in cancer, our goal is to define the force-induced protein-protein interactions surrounding the keratin network in vitro. The recombinant keratin 8 and keratin 18 were assembled into filaments, and the efficiency of keratin assembly was verified using ultra centrifugation. These filaments were deposited onto a glass coverslip and visualized using fluorescently labeled antibodies. To visualize stretch-dependent interactions, a microneedle was gently placed onto the keratin filaments and moved across the coverslip, thus pulling surface bound keratin fibers and straining them. Finally, purified cten proteins were added to the stretched keratin filaments. Interestingly, cten accumulated along both unstretched and stretched keratin filaments but more intensely along stretched keratin filaments, suggesting that cten binds keratin filaments directly in a force-dependent manner. We will further define the minimal force-sensitive sequence of cten and the role of this unique protein interaction between keratin and cten to understand the mechano-biology of keratins in the context of cancer.

Breakout Room: 5 - Gabriella Tzuwei Lai

OLFACTOMEDIN-LIKE 3 PROMOTES PROTUMOROGENIC GLIOMA-ASSOCIATED MICROGLIA

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Despite decades of research, glioblastoma multiforme (GBM) remains a uniformly lethal brain tumor. Transforming growth factor- β (TGF- β) in concert with glioma associated microglia (GAM), promotes GBM growth and invasion. Although TGF- β has a significant role in GBM progression, failed clinical trials suggest a complex role in GBM pathogenesis. Thus, an improved understanding of the TGF- β /GAM axis is critical to refine our therapeutic approach toward precise molecular targets. Olfactomedin-like 3 (OLFML3), a novel secreted glycoprotein, is elevated 9-fold in GBM. Although poorly studied within the context of GBM, OLFML3 contributes to tumor malignancy and angiogenesis in non-central nervous system cancer via promotion of tumor malignancy and neoangiogenesis. Importantly, Olfml3 is a direct target gene of TGF β 1 in mouse microglia. Our laboratory has demonstrated that Olfml3 is a direct target gene of all TGF β isoforms in mouse microglia (30-fold mRNA increase), but not glioma, nor brain endothelial cells. Given the profound immunosuppressive and pro-tumorigenic functions of TGF β signaling in glioma, we hypothesized that microglia derived Olfml3 polarizes microglia cells toward a pro-tumorigenic phenotype and promotes tumor cell growth kinetics. Using CRISPR/Cas9, we generated an Olfml3-knockout (Olfml3-/-) microglial cell line. Preliminary observations from our laboratory have demonstrated that microglia derived Olfml3 has intrinsic and extrinsic pro-tumorigenic effects. Loss of microglia Olfml3 attenuated secretion of several pro-tumorigenic molecules, including key factors promoting glioma growth and invasion

interleukin-1 and colony stimulating factor-1. Moreover, microglial secretion of CD95, a Fas ligand implicated in immune evasion, was undetectable in Olfml3 ^{-/-} media following exposure to TGFβ isoforms. Conditioned media from microglia treated with TGFβ induced neoangiogenesis in primary mouse brain endothelial cells and enhanced the migration and invasion of mouse glioma cell lines. Strikingly, these effects were abolished when microglia Olfml3 was deleted. In conclusion, microglia derived OLFML3 may contribute to glioma progression through multiple mechanisms, including promotion of the malignant glioma phenotype and neoangiogenesis. Further studies aim to elucidate microglia derived OLFML3 and its role in GBM progression in vivo.

Breakout Room: 6 - Ryan Toedebusch

INDUCTION OF CHROMATIN STRUCTURAL CHANGE IS A KEY DETERMINANT OF ANTI-TUMOR POTENCY OF THE NUCLEAR RECEPTOR RORγ ANTAGONISTS

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Nuclear hormone receptors (NRs) such as AR and ER are attractive targets of cancer therapeutics. We previously found that NR member ROR-γ is a novel therapeutic target in prostate cancer and triple-negative breast cancer (TNBC). We and others recently identified a number of structurally distinct antagonists/inverse agonists targeting ROR-γ and demonstrated their potency respectively in inhibition of tumor growth and in suppression of autoimmune disease-linked Th17 cell secretion of cytokines such as IL-17. However, there has not been any direct comparison between the different ROR-γ inhibitors in their cellular activities and underlying mechanism of action (MOA). Here, we showed that four different RORγ inhibitors displayed markedly different activities in inhibition of TNBC cell growth and survival. Our RNA-seq profiling revealed that antagonists XY018 and GSK805, but not VTP397 or TAK828F, strongly alter the expression of multiple major tumorigenic gene programs in TNBC cells. Among those gene programs, cholesterol biosynthesis pathway and EGFR-PI3K/Akt signaling pathway are significantly suppressed by XY018 and/or GSK805, but not by VTP397 or TAK828F. Our further mechanistic study using ATAC-seq revealed that XY018 but not VTP397 markedly reduces the chromatin accessibility at enhancer and/or promoter regions of genes involved in cholesterol biosynthesis pathway and the EGFR-PI3K/Akt signaling pathways. Together, our study provides for the first-time evidence that structurally distinct ROR-γ antagonists possess different activities in perturbing the function of their target ROR-γ in tissue/cell type-specific manner and that activities in altering chromatin structure is a key determinant of their effectiveness in blocking tumor growth.

Breakout Room: 7 - Hongye Zou

CERVICAL CANCER INCIDENCE AND STAGE AT DIAGNOSIS AMONG SENIORS ≥ 65 YEARS IN CALIFORNIA

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Background: Through screening, cervical cancer is detectable at early stage (stage I) disease. However, many patients in California present at late stage (stage II-IV) disease, especially seniors (≥ 65 years). Guidelines suggest discontinuing screening for seniors with a sufficient history of regular Pap tests who are not at high risk. However, prior studies show that compliance with cervical cancer screening guidelines decreases as patients approach age 65, and that cervical cancer incidence and late stage diagnosis in seniors is high with poor survival.

Purpose: To quantify the burden of cervical cancer diagnosis in patients ≥ 65 years and identify characteristics associated with late stage diagnosis.

Methods: Using California Cancer Registry data, we identified 13,570 patients diagnosed 2009-2018 with a first primary cervical cancer after age 21 years. Descriptive statistics compared the proportion of patients with late stage diagnosis by age group. Among patients ≥ 65 years, multivariable logistic regression estimated associations between sociodemographic and clinical characteristics with late stage diagnosis.

Results: One-fifth of patients ($n=2,429$, 20%) were ≥ 65 years. More seniors (64%) were diagnosed at late stage than any other age group followed by patients ages 50-64 (56%), 40-49 (42%), 30-39 (34%), and 21-29 (33%). Seniors in northern CA were 76% more likely to present with later stage disease than those in the Bay area (OR=1.76, CI 1.20-2.58). With each year of age, patients were 3% more likely to be diagnosed at late stage (OR=1.03, CI 1.01-1.04), and unmarried patients were 25% more likely to be diagnosed late stage (OR=1.25, CI 1.01, 1.53) than married patients. Patients diagnosed with adenocarcinoma were 29% less likely to be diagnosed at late stage than patients with squamous cell carcinoma (OR=0.73, CI 0.58-0.91). No association was found between year of diagnosis, health insurance type, neighborhood socioeconomic status, or race/ethnicity and late stage diagnosis.

Conclusions: Seniors in California were particularly vulnerable to late stage cervical cancer diagnosis. Our findings highlight the need to screen seniors with insufficient prior testing to prevent late stage cervical cancer diagnosis and associated mortality among seniors in California.

Breakout Room: 8 - Julianne J. P. Cooley

NUCLEAR RECEPTOR ROR- γ AND BRD4/BET BROMODOMAIN ANTAGONISTS SYNERGISTICALLY INHIBITS NEUROENDOCRINE DIFFERENTIATION IN PROSTATE CANCER

Xiong Zhang; Yang Yang and Hong-wu Chen

Neuroendocrine prostate cancer (NEPC) is a highly aggressive form of prostate cancer with a short survival time (typically < 12 months) from detection, arising either de novo or from prostate adenocarcinoma (PCA) treated with anti-androgen receptor (AR) signaling therapies. Tumor cell lineage plasticity such as aberrant transition to NE is now recognized as a major mechanism that confers resistance to most of the current therapies. Epigenetic alterations in the tumors have been strongly implicated. However, so far only a few drivers and therapeutic targets of NEPC diseases have been identified. Here, we report that through small-molecule perturbation of major epigenetic regulators, we identified chromatin regulator BRD4 and nuclear receptor ROR- γ as strong candidates of therapeutic targets in NEPC. In a limited screening of compounds that perturb the function of epigenetic regulators, we found that inhibitors of BRD4 displayed strong activities in suppression of NEPC cell growth. Among them, BRD4 inhibitor AZD5153, which is currently on clinical trial for released solid tumors and lymphoma, showed the highest potency. Epigenetic regulator BRD4 activates gene expression through binding to acetylated nucleosome histones by its bromodomains. Interestingly, we observed that combinations of AZD5153 with ROR- γ antagonist GSK805 and XY018 resulted in strong synergies in inhibition of the NEPC cell growth and survival. Our RNA-seq gene expression profiling demonstrated that among genes downregulated by the treatments, neuroactive ligand-receptor interaction and cAMP signaling pathways are significantly enriched. Further analyses validated that indeed key components of the pathways such as phosphorylation of cAMP-dependent protein kinase (PKA) and its downstream transcription factor cAMP-response element binding protein (CREB), as well as the NEPC markers (e.g. SYP, CHGA and ENO2) were strongly down-regulated by the inhibitor treatments. Next, we conducted Transposase Accessible Chromatin sequencing (ATAC-seq) to analysis genome wide changes in open and closed chromatin. The combination of XY018 and AZD5153 led to a dramatically genome-wide decrease in open chromatin as well as the closing of peaks in neurogenic genes and CREB controlled genes. Therefore, our study identified a combinatorial targeting of epigenetic regulator BRD4 and nuclear receptor ROR- γ as a promising, new strategy for treatment of NEPC.

Breakout Room: 9 - Xiong Zhang

PERFORMANCE OF HIGH-RESOLUTION MICRO-ULTRASOUND FOR DIAGNOSING CLINICALLY SIGNIFICANT PROSTATE CANCER IN MEN UNDERGOING A SCREENING MRI

Aman Arora, Neil Pugasehetti MD, Christopher P Evans MD, Marc A Dall'Era MD

Introduction: Prostate cancer is one of the most common cancers, affecting up to 11% of men over their lifetime. The diagnosis can be challenging, as most men with early prostate cancer are asymptomatic and diagnosed based on PSA screening. The standard technique for prostate biopsy (bx) is with transrectal ultrasonography (US) (6-9 MHz) and either a transrectal or transperineal needle approach. Technological advances have produced a high frequency (29MHz) transrectal prostate micro-ultrasound system with up to 70 μ m resolution, allowing for finer visibility of ductal anatomy and cellular density. In this study, we examined our initial experiences with high frequency micro-ultrasound machine for the detection of prostate cancer after mpMRI.

Methods: With IRB approval, we retrospectively examined medical records of 51 patients at UC Davis Medical Center who underwent a prostate mpMRI followed by transrectal bx using the high-frequency US system (Exact Imaging, Toronto, Canada). On and off target cores were taken with MRI lesions targeted using the integrated fusion assist targeting system. The primary outcome was prostate cancer detection rate stratified by cancer grade group (GG) and PIRADS score. A secondary outcome was detection of GG ≥ 2 prostate cancer stratified by PIRADS score.

Results: Seventy-one percent of patients had a positive bx for carcinoma, with 51% of patients having GG ≥ 2 disease. Positive bx rates by PIRADS score were 60%, 76%, and 93% for PIRADS score 3, 4, and 5, respectively. 81% of patients with GG ≥ 2 had a PIRADS ≥ 3 lesion on MRI. Forty-two percent of patients with a negative mpMRI (PIRADS ≤ 2) had a positive bx, with 80% of them having GG ≥ 2 disease. The negative bx rate decreased with increasing PIRADS score, from 58% with a PIRADS ≤ 2 , to 7% with a PIRADS 5 lesion.

Conclusion: Our study demonstrated good performance of a high-resolution ultrasound system after MRI for prostate cancer detection. The negative bx rate dropped substantially with higher PIRADS score and findings are consistent with other series describing technology for MRI lesion targeting.

Breakout Room: 10 - Aman Arora

THE INHIBITORY EFFECT OF ECG AND EGCG DIMERIC PROCYANIDINS ON COLORECTAL CANCER CELLS GROWTH IS ASSOCIATED WITH THEIR ACTIONS AT LIPID RAFTS AND THE INHIBITION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR SIGNALING

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Colorectal cancer (CRC) is one of the most common cancers worldwide. Epidemiological studies indicate that consumption of fruits and vegetables containing procyanidins is associated with lower CRC risk. This study investigated the capacity of two dimeric procyanidins composed of epicatechin gallate (ECG) or epigallocatechin gallate (EGCG) isolated from persimmons, to inhibit CRC cell growth and promote apoptosis, characterizing the underlying mechanisms. ECG and EGCG dimers reduced the growth of five human CRC cell lines in a concentration (10–60 μ M)- and time (24–72 h)-dependent manner, with a 72 h-IC₅₀ value in Caco-2 cells of 10 and 30 μ M, respectively. ECG and EGCG dimers inhibited Caco-2 cell proliferation by arresting the cell cycle in G2/M phase and by inducing apoptosis via the mitochondrial pathway. In addition, ECG and EGCG dimers inhibited cell migration, invasion, and adhesion, decreasing the activity of matrix metalloproteinases (MMP-2/9). Mechanistically, ECG and EGCG dimers inhibited the activation of lipid raft-associated epidermal growth factor (EGF)

receptor (EGFR), without affecting its localization at lipid rafts. In particular, ECG and EGCG dimers reduced EGFR phosphorylation at Tyr1068 residue, prevented EGFR dimerization and activation upon stimulation, and induced EGFR internalization both in the absence and presence of EGF. Furthermore, ECG and EGCG dimers increased EGFR phosphorylation at Tyr1045 residue, providing a docking site for ubiquitin ligase c-Cbl and induced EGFR degradation by the proteasome. Downstream of EGFR, ECG and EGCG dimers inhibited the activation of the MEK/ERK1/2 and PI3K/AKT signaling pathways, downregulating proteins involved in the modulation of cell survival. In conclusion, ECG and EGCG dimers reduced CRC cell growth by inhibiting EGFR activation at multiple steps, including the disruption of lipid rafts integrity and promoting EGFR degradation. These results shed light on a potential molecular mechanism on how procyanidins-rich diets may lower CRC risk.

Breakout Room: 11 - Wei Zhu