2024 OCMF

Est. 2019

Ovarian Cancer Midwest Focus Conference

Cancer CoBRE Symposium

SANFORD RESEARCH

Conference Location:
Sanford Research Center
The Dakota Room
2301 East 60th St N
Sioux Falls, South Dakota

University of Minnesota
Department of OBGYN &
Women's Health

2024 CMF Ovarian Cancer Midwest Focus Conference
Est. 2019

SANFORD RESEARCH

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2024 OCMF
Est. 2019
Ovarian Cancer Midwest Focus Conference

Welcome

Welcome to the 6th annual Minnesota-based international Ovarian Cancer Midwest Focus (OCMF) Conference! This year we have merged with our hosting institution, Sanford Research, and its annual Cancer CoBRE symposium.

Program Chairs

Martina Bazzaro, PhD
University of Minnesota, School of Medicine

Ronny Drapkin, MD, PhD
University of Pennsylvania - Perelman School of Medicine

Andrew K. Godwin, PhD
University of Kansas Medical Center - Kansas Institute for Precision Medicine

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Sanford Research and University of South Dakota

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2024 OCMF
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Ovarian Cancer Midwest Focus Conference

Day 1

NOVEMBER 18, 2024

Breakfast: 7:15 AM - 8:00 AM

SESSION 1: CANCER CHROMOSOMES

MODERATORS: MARTINA BAZZARO, PHD & ANDREW K. GODWIN, PHD

TIME	TITLE	PRESENTER
8:00 - 8:10 AM	Opening Remarks and Welcome	
8:10 - 9:00 AM	Evolution of Genome Instability in Cancer	**KEYNOTE SPEAKER** **Don Cleveland, PhD** University of California, San Diego
9:00 - 9:10 AM	Question & Answer Session	
9:10 - 9:30 AM	Cell Dynamics in the Omentum: Insights Into Pre-metastatic Niche Formation and Metastasis Prevention	**Honami Naora, PhD** University of Texas, MD Anderson Cancer Center
9:30 - 9:50 AM	The Biology of Stress and Resilience in Ovarian Cancer	**Susan Lutgendorf, PhD** University of Iowa
9:50 – 10:00 AM	Question & Answer Session	
10:00 - 10:10 AM	BREAK	
10:10 - 10:30 AM	Early Detection in Ovarian Cancer: The Role of miRNAs	**Kevin Elias, MD** Cleveland Clinic
10:30 - 10:50 AM	The Aging Ovary and Ovarian Cancer Risk	**Barbara Vanderhyden, PhD** University of Ottawa
10:50 – 11:00 AM	Question and Answer Session	

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Day 1

NOVEMBER 18, 2024

SESSION 1: CANCER CHROMOSOMES

MODERATORS: MARTINA BAZZARO, PHD & ANDREW K. GODWIN, PHD

TIME	TITLE	PRESENTER
Awards Session Presenters: Ronny Drapkin, MD, PhD; Paola Vermeer PhD; Mayor Paul TenHaken		
11:05 - 11:30 AM	Award for Outstanding Support for Ovarian Cancer Research	**Tina's Wish** Received by Beverly Wolfer
	Inspiration and Philanthropy Award	**Denny Sanford** Received by Kyle Roux
	Award for Excellence in Community Outreach	**Sanford Promise Lab** Received by Tamara Ledeboer, Louisa Otto, Ben Benson
Abstract Flash Talks		
11:30 - 12:10 PM	Parallel Epigenetic and Transcriptomic Profiling of Carcinoma and Stroma Compartments Across Major Ovarian Cancer Histotypes	**Svetlana Djirackor, PhD Candidate** Van Andel Institute
	Inhibition of Glutamine Metabolism Suppresses Tumor Progression Through Remodeling of the Macrophage Immune Microenvironment	**Tian-Li Wang, PhD** Johns Hopkins University School of Medicine
	Multimodal Spatial Profiling Reveals Immune Suppression and Microenvironment Remodeling in Fallopian Tube Precursors to High-Grade Serous Ovarian Carcinoma	**Tanjina Kader, PhD** Harvard Medical School

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TIME	TITLE	PRESENTER
11:30 - 12:10 PM	Adrenergic Signaling in Adipocytes Drives Ovarian Cancer Cell Invasion	**Monica Haughan, PhD Candidate** University of Illinois Cancer Center
12:10 - 12:20 PM	Flash Talk Question & Answer	
12:20 - 2:00 PM	**Lunch Break** Poster Sessions in the East Bay Sponsor & Exhibitor Presentations in the Dakota Room	

SESSION 2: OVARIAN CANCER MICRO ENVIRONMENT

MODERATORS: RONNY DRAPKIN, MD, PHD & BORIS WINTERHOFF, MD, MS

TIME	TITLE	PRESENTER
2:00 - 2:20 PM	Metabolic Alterations in Neurofibromatosis Type 1	**Seth Tomchik, PhD** University of Iowa
2:20 – 2:40 PM	A Brain-body Feedback Loop Driving HPA-axis Dysfunction in Breast Cancer	**Jeremy Borniger, PhD** Cold Spring Harbor Laboratory
2:40 - 2:50 PM	Question & Answer Session	
2:50 - 3:10 PM	Single-cell Analysis of Fallopian Tube Immune Cells and Implications for Early Cancer Detection	**Huy Q. Dinh, PhD** University of Wisconsin

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SESSION 2: OVARIAN CANCER MICRO ENVIRONMENT

MODERATOR: RONNY DRAPKIN, MD, PHD & BORIS WINTERHOFF, MD

TIME	TITLE	PRESENTER
3:10 - 3:30 PM	The Influence of the Tumor Microenvironment in Ovarian Cancer Development and Metastasis	**Hilary Kenny, PhD** University of Chicago
3:30 - 3:40 PM	Question & Answer Session	
3:40 - 4:10 PM	Sponsor & Exhibitor Presentations	
4:10 - 4:15 PM	Closing Remarks	

RECEPTION

Monday, November 18

The Barn, 2510 East 54th St. N, Sioux Falls, SD

4:30 PM - 9:00 PM

Join us for dinner, drinks, and fun at Casino Night! Transportation will be waiting to take you to The Barn for dinner, drinks and games!

Transportation will also be available back to the two conference hotels.

2024 Est. 2019 OCMF Ovarian Cancer Midwest Focus Conference

Day 2

NOVEMBER 19, 2024

Breakfast: 7:15 AM - 8:00 AM

SESSION 3: TRANSLATING INNOVATION TO THE CLINIC

MODERATOR: GOTTFRIED E. KONECNY, MD & PAOLA VERMEER, PHD

TIME	TITLE	PRESENTER
8:00 - 8:10 AM	Opening Remarks & Welcome	
8:10 - 8:40 AM	Patient Advocates	
8:40 - 9:30 AM	RaceCAR-Macrophage Therapy: Harnessing Cellular Cannibalism to Combat Cancer	**KEYNOTE SPEAKER** **Denise Montell, PhD** University of California, Santa Barbara
9:30 - 9:40 AM	Question & Answer Session	
9:40 - 10:00 AM	The Next Generation in Immunotherapy: Ovarian Cancer	**Steven Powell, MD, FASCO** Sanford Research
10:00 - 10:20 AM	Preclinical Study of a Novel Vaccine Against Head and Neck Cancer	**Wenfeng An, PhD, MPH** South Dakota State University
10:20 – 10:30 AM	Question & Answer Session	
10:30 - 10:40 AM	BREAK	
10:40 – 11:00 AM	Identifying Novel Pathways of SCLC Progression	**Michael Kareta, PhD** Sanford Research

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SESSION 3: TRANSLATING INNOVATION TO THE CLINIC

MODERATOR: GOTTFRIED E. KONECNY, MD & PAOLA VERMEER, PHD

TIME	TITLE	PRESENTER
11:00 - 11:20 AM	Metabolic Labeling of Nascent Extracellular Matrix in Physiologically Relevant Preclinical Models	**Pilar de la Puente, PhD** Sanford Research
11:20 - 11:40 AM	Point-of-care Assessment of Anticancer Drug Dosage for Evaluating the Efficacy of Cancer Treatment	**Xiaojun Xian, PhD** South Dakota State University
11:40 - 11:50 AM	Question & Answer Session	
Abstract Flash Talks		
11:50 - 12:30 PM	Single Cell Epigenomic Characterization of Transposable Elements in High Grade Serous Ovarian Cancer	**Yiwen Xie, PhD Candidate** Mayo Clinic
	Turning Hypoxic Immunologically "Cold" Ovarian Tumors into "Hot" Tumors: Reprogramming the Tumor-Immune Microenvironment	**Simona Plesselova, PhD** Sanford Research
	AXL Receptor Tyrosine Kinase, a Potential Biomarker and Therapeutic Target of Ovarian Cancer	**Li Wang, Postdoc** Mayo Clinic
	Preclinical Evaluation of UMN:SS101 as an Advanced Ovarian Cancer Therapy	**Joshua Krueger, PhD Candidate** University of Minnesota

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TIME	TITLE	PRESENTER
12:30 - 12:45 PM	Flash Talk Question & Answer	
12:45 - 1:30 PM	Flash Talk Awards, Closing Remarks, and Lunch To-Go	

Thank You

On behalf of the OCMF Program Chairs, we would like to express our sincere gratitude to this year's sponsors, speakers and participants.

As we continue the fight against ovarian cancer, your contributions and collaboration are vital in our effort to expand education for those involved at all levels of research.

Please join us next year!
Dates and location will be announced soon.

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Flash Talk Abstracts

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Inhibition of Glutamine Metabolism Suppresses Tumor Progression through Remodeling of the Macrophage Immune Microenvironment

Tianhe Li, Sepehr Akhtarkhavari, Ie-Ming Shih, Stephanie Gaillard, **Tian-Li Wang**
Johns Hopkins University School of Medicine

Targeting glutamine metabolism has emerged as a promising strategy in cancer therapy. However, several barriers, such as in vivo anti-tumor efficacy, drug toxicity, and safety, remain to be overcome to achieve clinical utility. Prior preclinical in vivo studies had generated encouraging data showing promises of cancer metabolism targeting drugs although, most were performed on immune-deficient murine models. Apart from tumor cells, normal cells such as immune cells in the tumor microenvironment may also utilize glutamine for maintaining physiological functions. Hence, the current study employed two murine models of gynecological cancers to evaluate the impact of glutamine antagonists on tumor and immune microenvironment. Moreover, we developed a glutamine antagonist prodrug, JHU083, which is bio-activated restrictively in cancer tissues to enhance safety and reduce potential off-target effects, Applying this prodrug in vivo allows an in-depth view of glutamine metabolism dependency in cells within the tumor microenvironment. In both murine tumor models, we observed significant anti-tumor efficacy, resulting in reduced tumor burden and impeded tumor progression. Single-cell RNA sequencing performed on tumor tissues revealed significantly impeded immunosuppressive M2-like macrophages by JHU083, while the treatment spared pro-inflammatory M1-like tumor macrophages. In many TME cells, JHU083 downregulated genes regulated by Myc and hypoxia. M2 macrophages' higher sensitivity to glutamine antagonism compared to M1 macrophages or monocytes was further validated in ex vivo bone marrow-derived cultures. Taken together, our findings indicate that JHU083 exerts its anti-tumor efficacy not only through direct targeting of glutamine-addicted cancer cells directly, but also by promoting an immune-stimulatory microenvironment within the M1/M2 macrophages.

Parallel Epigenetic and Transcriptomic Profiling of Carcinoma and Stroma Compartments Across Major Ovarian Cancer Histotypes

Svetlana Djirackor, Karolin Heinze, Ian Beddows, David Sokol, Bianca Ribeiro de Souza, Martin Koebel, Marie Adams, Michael Anglesio, Hui Shen
Van Andel Institute

The molecular landscapes of epithelial ovarian cancers (EOCs) have largely been profiled using bulk tumor samples. Biologically relevant signals from the tumor and its microenvironment (stroma) are often conflated, dampening both tumor and stroma-specific observations. Here, we profiled the carcinoma and stroma compartments separately across clear cell, endometrioid, and high-grade serous ovarian cancer using laser capture microdissection followed by sample-matched whole genome bisulfite sequencing and RNA-seq. Across these histotypes, stromal DNA methylation shows minimal variation with only 11 differentially methylated regions (DMRs). In contrast to DNA methylation, the stromal compartment displayed extensive subtype-specific gene expression programs. This suggests potentially reversible local education rather than epigenetically coded, mitotically heritable, cellular memories in the stroma. In addition, unsupervised clustering of the top variable genes defined 5 stromal subtypes shared across the three EOCs, with one subtype characterized by pronounced myofibroblast presence. Coupling the stromal subtypes with the well-established histotype designations of EOCs may provide a more robust way to classify EOCs and inform optimal treatment strategies that account for the characteristic of the tumor stroma.

Turning Hypoxic Immunologically "Cold" Ovarian Tumors into "Hot" Tumors: Reprogramming the Tumor-Immune Microenvironment

Simona Plesselova, Pilar de la Puente
Sanford Research

Ovarian cancer (OC) has the highest mortality to incidence ratio among all gynecological cancers with recurrence and resistance to chemotherapy and immunotherapy in more than 80% of patients in advanced stage. Paradoxically, OC was one of the first cancers where the presence of tumor-infiltrated lymphocytes (TILs) was correlated with better immunotherapeutic response and survival rate. OC is characterized by hypoxia (low O2 levels) which dictates an immunologically cold tumor microenvironment (TME) with enhanced immunosuppressive signals and cell types and activation of cancer-associated fibroblasts (CAFs). Activated CAFs enhance collagen secretion through TGF-β signaling leading to increased stiffness of the extracellular matrix (ECM) that forms a physical barrier for immune infiltration and immunotherapy. Despite the physiologically relevant O2 levels in the healthy ovary being about 7% O2, current studies compare the hypoxic TME (1.5% O2) with hyperoxic culture models (21% O2). Therefore, there is a pressing need to establish tissue biomimetic models to recapitulate malignant hypoxic and healthy physoxic ovarian environments and identify new therapeutic targets to turn hypoxic immunologically-cold tumors into hot ones.

A patient-derived physiologically relevant 3D model was bioengineered to mimic the physoxic levels of the healthy ovary (3D-Physoxia) and the hypoxic O2 levels of the OC-TME (3D-Hypoxia) by modulating matrix height, crosslinking, and exposure to externally controlled O2 levels. This model is based on crosslinking of human plasma fibrinogen into fibrin, to form personalized 3D model for culture of patient-matched immune cells with cancer cells and CAFs from OC tumor biopsies mimicking the OC-TME. Immune cells were added on top of the 3D model for immune infiltration assays, or co-cultured inside the matrix to study evasion mechanisms once they penetrated the OC-TME. Flow cytometry was used to detect infiltrated immune cells and activation and exhaustion markers to validate their functionality. Galunisertib, a TGF-β1 receptor inhibitor, was used to overcome CAFs activation, ECM remodeling and immunosuppressive TME.

Continued...

Turning Hypoxic Immunologically "Cold" Ovarian Tumors into "Hot" Tumors: Reprogramming the Tumor-Immune Microenvironment

Simona Plesselova, Pilar de la Puente
Sanford Research

The 3D model recapitulated the physiologically relevant hypoxic O2 level in OC (0.7% O2, 3D-Hypoxia) when incubated in 1.5% O2 incubator and the physoxic healthy ovary O2 level (7% O2, 3D-Physoxia) when incubated in 21% O2. Hypoxic status, an enhanced expression of collagen I, and significantly higher stiffness were confirmed in 3D-Hypoxia compared to 3D-Physoxia. Significantly impaired infiltration of CD8+ T cytotoxic cells into the coculture of OC with CAFs was observed by flow cytometry in 3D-Hypoxia compared to 3D-Physoxia and was rescued by pre-treatment with TGF-β inhibitor galunisertib, suggesting an implication of CAFs in ECM remodeling and immune infiltration through TGF-β signaling. Moreover, CD8+ cells expressed higher levels of cytolytic enzyme Granzyme B and had higher cytotoxic effect on OC cells in 3D-Hypoxia compared to 3D-Physoxia. Also, hypoxia induced significantly enhanced expression of activation markers CD69, intracellular IL-2, and secreted IFNγ in CD8+ cells compared to 3D-Physoxia, but also a higher immunosuppressive Treg population. Critically, these data suggest that hypoxia acts as a friend and foe in the OC-TME by enhancing CD8 activation and cytotoxicity to kill the tumor cells but conversely also increasing an immunosuppressive TME with ECM remodeling impairing immune infiltration into the tumor. In conclusion, we have engineered and functionally characterized a physiologically relevant 3D model to study the role of hypoxia and ECM remodeling on tumor-immune interactions. Our results are expected to have a positive impact because they will shed light on how to improve immunotherapy efficacy and activate the immune system by turning immunologically cold tumors into hot ones targeting the dysregulated ECM remodeling via TGF-β signaling.

Multimodal Spatial Profiling Reveals Immune Suppression and Microenvironment Remodeling in Fallopian Tube Precursors to High-Grade Serous Ovarian Carcinoma

Tanjina Kader, Jia-Ren Lin, Clemens Hug, Shannon Coy, Yu-An Chen, Ino de Bruijn, Roxanne J. Pelletier, Mariana Lopez Leon, Jong Suk Lee , Clarence Yapp, Natalie Shih, Euihye Jung, Baby Anusha Satravada, Ritika Kundra, Yilin Xu, Sabrina Chan, Julian B. Tefft, Jeremy Muhlich, Gabriel Mingo, Dalia Omran, Judith Agudo, Nikolaus Schultz, Charles Drescher, Peter K Sorger, Ronny Drapkin, Sandro Santagata

Harvard Medical School

High-Grade Serous Ovarian Cancer (HGSOC) originates from fallopian tube (FT) precursors. To characterize molecular features during HGSOC development, we integrated high-plex imaging and spatial transcriptomics of FT precancer lesions, including p53 signatures, serous tubal intraepithelial carcinomas (STIC), and invasive HGSOC. Our data reveal immune modulating mechanisms within precursor epithelium, characterized by chromosomal instability, persistent interferon (IFN) signaling, and dysregulation of innate and adaptive immunity. FT precursors display increased expression of MHC-class I, including HLA-E, and IFN-stimulated genes, typically linked to later-stage tumorigenesis. The microenvironment shifts along the progression axis from immune surveillance with NK cells, type 1 conventional dendritic cells, and CD8+ T cells in p53 signatures and early STICs to increasing immune suppression in cancer and associated STICs, characterized by regulatory T cells, macrophages, and immune dysfunction. These findings highlight potential biomarkers and therapeutic targets for intercepting HGSOC and provide resources to study molecular transitions from precancer to cancer.

Adrenergic Signaling in Adipocytes Drives Ovarian Cancer Cell Invasion

Monica A. Haughan, Hannah J. Lusk, Carlismari Grundmann, Tova M. Bergsten, Aloysius J. Klingelhutz, Laura M. Sanchez, and Joanna E. Burdette
University of Illinois Chicago

High grade serous cancer (HGSC), the most common and lethal ovarian cancer subtype, can arise from the fallopian tube and habitually metastasizes to the omentum. We employ murine oviductal epithelial (MOE) cells expressing PTEN shRNA as a model of fallopian tube derived HGSC. To uncover the metabolic crosstalk in HGSC omental metastasis, we leverage imaging mass spectrometry (IMS) which allows us to visualize the chemistry produced in co-cultures of HGSC cells and omental explants. IMS identified several metabolites, including epinephrine, as unique to the MOE PTENshRNA + omentum condition. We hypothesize autocrine epinephrine signaling in omental adipocytes liberates fatty acids driving tumor progression. To investigate this mechanistically, we utilize a human pre-adipocyte line that forms spheroids and can differentiate in culture. IMS of adipocytes spheroids alone and in co-culture within MOE PTENshRNA revealed epinephrine production confirming the omental cell type responsible was adipocytes. Conditioned media (CM) was collected from murine omental explants and adipocyte spheroids treated with epinephrine (10mM), propranolol (1mM), or vehicle. MOE PTENshRNA, OVCAR4, and OVCAR8 were treated with these CM to investigate the impact on invasion via Boyden chamber assay. CM from both adipocyte sources treated with propranolol significantly decreased invasion compared to vehicle, while only epinephrine treated omental CM significantly increased invasion of MOE PTENshRNA. Further we performed untargeted lipidomics and uncovered several unique ions exclusive to the epinephrine treated CM including glycerolipids, glycerophospholipids, and sphingolipids. Current work seeks to determine if exogenous addition of identified lipids is sufficient to drive HGSC cell invasion. Overall, we seek to understand the mechanisms of omental metastasis and impact of the epinephrine identified by IMS.

Single Cell Epigenomic Characterization of Transposable Elements in High Grade Serous Ovarian Cancer

Yiwen Xie, M.S., Wazim Mohammed Ismail, Ph.D., Amelia Mazzone, Ph.D., Leticia Sandoval, M.D., Megan Ritting, Musheer Alam, Ph.D., Nagarajan Kannan, Ph.D., M.S., Saravut (John) Weroha, M.D., Ph.D., Katherine Chiappinelli, Ph.D., Alexandre Gaspar-Maia, Ph.D.
Mayo Clinic

High-grade serous carcinoma (HGSC) is the most prevalent and lethal subtype of the epithelial ovarian cancers and the focus of this study. Transposable Elements (TEs) are DNA sequences that can translocate between chromosomal locations. Previous research has shown that TEs including the long-interspersed element 1 (LINE1) are abnormally enriched in HGSC. However, it remains unclear how the TEs global expression landscape and functions are affected by the standard of care chemotherapy regimen. In this study, we performed the single cell multi-omics sequencing (scATAC-seq and snRNA-seq from the same cells) using the patient tissues from 5 individuals with untreated naïve HGSC, 3 individuals with neoadjuvant chemotherapy treated HGSC, and 5 individuals with non-malignant fallopian tubes (FT). We applied a TE-specific pipeline to evaluate the TEs expression features across each group and integrated the multiomics data with the ChIP-seq database to characterize the epigenetic regulatory landscape of TEs in HGSC following neoadjuvant chemotherapy. Strikingly, our analysis reveals that retrotransposons, including LINEs and long terminal repeats (LTRs), are globally dysregulated by neoadjuvant treatment. The cell type-specific analysis suggests that epithelial cells display the top differentially expressed TEs across the heterogeneity. Interestingly, we also observe significant changes in TEs expression in other cell types from the tumor microenvironment, suggesting the crosstalk between multiple subtypes. Moreover, we validated that the decreased LINEs and LTRs by neoadjuvant chemotherapy consistently suppresses the inflammatory responses in HGSC by the single-cell GSVA analysis. Epigenomic (scATAC-seq) analysis of the TEs loci additionally revealed the enrichment of the TEs is associated with the loci-specific hypomethylation but may not be solely driven by the DNA methylation patterns. Indeed, we found the enrichment of the TEs in HGSC is associated with the loci-specific hypomethylation, and there exist a unique dependency of the upregulated retrotransposons on the FOX transcription factors (TFs) family in HGSC. Altogether, our data lend support to the hypothesis that epigenetic alteration is a major regulatory mechanism of TEs activation, which is disrupted by chemotherapy, further identifying TEs as the potential markers for HGSC progression therapeutic response prediction.

AXL receptor tyrosine kinase, a potential biomarker and therapeutic target of ovarian cancer

Li Wang, Jidong Wang, Md Kamrul Hasan Khan, Nicole A. Pearson, Yajue Huang, Hunter J. Atkinson, Melissa C. Larson, Sun-Hee Lee, Xiaonan Hou, Akhilesh Pandey, Jamie N. Bakkum-Gamez, Ann L. Oberg, Scott H. Kaufmann, John J. Weroha, Xinyan Wu

Mayo Clinic

High-grade serous ovarian cancer (HGSOC) represents the predominant histological subtype of ovarian cancer, accounting for the majority of related mortality. The challenges of effective early detection and therapeutic resistance significantly impact patient outcomes. AXL, a receptor tyrosine kinase of TAM family, plays critical roles in cancer progression and treatment resistance and is emerging as a potential biomarker and therapeutic target.

Our study demonstrates that AXL is overexpressed in 80% of HGSOC patients and serves as an independent predictor of poor progression-free survival (p=0.009) and short overall survival (p=0.010), highlighting its therapeutic relevance in AXL-positive cases. Additionally, we identified soluble AXL (sAXL), a proteolytically cleaved form of AXL's extracellular domain that can be detected in patients' plasma, significantly correlates with tumor debulking status (p=0.002), poorer overall survival (p=0.002) and progression-free survival (p=0.009). More importantly, in patients with optimal debulking status, sAXL level is associated with poorer prognosis (p=0.018), suggesting sAXL as a better prognosis marker than debulking status.

To elucidate AXL's oncogenic function and therapeutic potential, we generated AXL-inducible knockout and overexpression cell lines using the HGSOC cell line OVCAR8. Our results indicate that cell proliferation and colony formation are positively correlated with AXL levels. In vivo studies revealed that AXL-overexpressing cells exhibit accelerated growth, while endogenous AXL knockout significantly inhibits tumor growth. Consistent with these data, we found that AXL siRNA and inhibitors have a universal effect on AXL-expressing ovarian cancer cell lines but not on AXL-null cell lines.

To disentangle the downstream signaling of AXL and clarify its regulatory mechanisms in HGSOC, we employed mass spectrometry-based quantitative proteomic and phospho-proteomic analysis to map the downstream signaling pathways regulated by AXL suppression via siRNA. Pathway enrichment analysis revealed that suppressing AXL expression could inhibit multiple important cancer-related signaling pathways, including cell cycle, focal adhesion and MAPK signaling pathways.

This will enhance our understanding of the oncogenic role of AXL in ovarian cancer progress and how ovarian tumors respond to AXL inhibition treatment in HGSOC patients, which is crucial for designing effective clinical trials and refining response signatures to facilitate patient stratification.

Preclinical Evaluation of UMN:SS101 as an Advanced Ovarian Cancer Therapy

Joshua B. Krueger, Jae-Woong Chang, Alexandria Gilkey, Timothy D. Folsom, Ethan Niemeyer, Joseph G. Skeate, Melissa Geller, Beau R. Webber, and Branden S. Moriarity
University of Minnesota

Natural killer (NK) cells have been engineered to express chimeric antigen receptors (CARs) in an effort to combine their innate cytotoxicity with CAR-mediated activation. This strategy has shown notable success against hematologic cancers in clinical trials (1), however, its application to solid tumors has been limited, largely due to challenges in CAR-NK cell expansion, persistence, and achieving sufficient tumor clearance in vivo. Last year, we developed an armored CAR-NK therapy aimed at targeting mesothelin-positive ovarian cancers (Figure 1A). We tested three anti-mesothelin binding domains, and although no significant differences were observed in vitro (Figure 1B), an in vivo pilot study where 5e6 A1847 cells were injected intraperitoneally [IP] on day 0 and 5e6 armored CAR NK cells administered IP on day 14, identified the SS1 binding domain to be the strongest option. (Figure 1C,D). This led us to prioritize SS1 for further testing. Building on this, we aimed to enhance the innate killing potential of the NK cells beyond their baseline activity. This resulted in the development of UMN:SS101 a final product incorporating the CAR, soluble IL-15, and small molecule reprogramming during NK cell expansion to further boost cytotoxicity. When tested in a challenging ovarian cancer model (5e6 A1847 cells injected IP on day 0 and a reduced dose of 5e5 therapy cells on day 7), UMN:SS101 achieved complete tumor clearance (Figure 2A,B) and showed high NK cell counts in the blood, bone marrow, and intraperitoneal space (Figure 2C). Despite these promising results, the treated mice had a shorter survival (~30 days) compared to the tumor-only control group (~55 days) (Figure 2D). We hypothesized that this was due to the NSG mouse model's inability to fully mimic the complex immune interactions found in human patients. While NSG mice offer advantages such as high tumor engraftment and therapeutic persistence, they lack an intact immune system, which might have led to unregulated expansion of UMN:SS101 causing early death in mice, a phenomenon noted by other CAR-NK groups (2,3,4). In a clinical setting, patients receiving allogeneic NK therapies retain immune function, which suppresses non-donor matched NK cells, potentially mitigating this effect (5). To better simulate an immunocompetent environment and further validate UMN:SS101, we developed a humanized mouse model by injecting MHC double-knockout mice with human peripheral blood mononuclear cells (PBMCs). In this model 2.5e6 OVCAR8 cells injected on day 0, 10e6 PBMCs on day 1, and 5e5 therapy cells on day 7, (Figure 3A) UMN:SS101 significantly extended survival, with two out of three treated mice surviving until the study's endpoint (Figure 3B). Notably, the single mouse that did not benefit from the therapy also failed to achieve effective humanization with PBMCs, thereby replicating the earlier NSG study (Figure 3C). A second dose of UMN:SS101 at week 9 confirmed its efficacy, as a renewed therapeutic response was observed (Figure 4D). These results show the potential of UMN:SS101 as a safe and potent off-the-shelf therapy. We are now preparing for a phase 1 clinical trial, with ongoing safety assays including independent growth evaluations, off-target killing analysis, and further in vivo validations. We are driven to provide physicians and patients with a safe and effective option when looking to CAR-NK therapy. A note to the evaluators: The "small molecule reprogramming" is intentionally vague as we are nearing publication and would like to reveal the specifics at that time.

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Poster Session Abstracts

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Veratridine, a potent mTORC2 inhibitor: A promising platform for anti-metastatic drug discovery

Morgan Eikanger, Dr. Khosrow Rezvani
University of South Dakota

Despite considerable advances to improve colorectal cancer (CRC) survival over the last decade, therapeutic challenges remain due to the rapid metastatic dissemination of primary tumors and screening limitations. Meanwhile, the rise of CRC in younger adults (early-onset CRC), commonly diagnosed with a metastatic form of the disease, shows the pressing need to develop more effective targeted therapies capable of decreasing the high mortality rates associated with metastatic CRC. Hyperactivation of the Rictor-mTORC2-AKT signaling pathway drives key metastatic players in diverse malignant tumors, including early- and late-onset CRC. Selective mTORC2 inhibitors are becoming a potential optimal treatment strategy for patients with CRC because it eliminates therapeutic limitations and drug resistance reported for mTORC1 and mTORC1/mTORC2 inhibitors. Veratridine (VTD), a lipid-soluble alkaloid extracted from Liliaceae plants, can transcriptionally increase UBXN2A, which induces 26S proteasomal degradation of the Rictor protein, a key member in the mTORC2 complex. Destabilization of Rictor protein by VTD decreases metastatic signaling downstream of the mTORC2 pathway in diverse malignant tumors. This study showed a novel mechanistic connection between a ubiquitin-like protein and mTORC2-dependent migration and invasion in CRC tumors. In a UBXN2A-dependent manner, VTD suppresses colon cancer cell invasiveness and the ensuing risk of tumor metastasis by decreasing the population of metastatic colon cancer stem cells. VTD can effectively restrict cell migration during real-time cell analysis (RTCA) and in a three-dimensional (3D) spheroid CRC model. This study opened a new platform for repurposing VTD, a supplemental anti-hypertension molecule, into an effective targeted therapy in CRC tumors.

Proteomic profiling of the effects of NB compound FOXM1 inhibitors in high-grade serous ovarian carcinoma

Makenzie Vorderbruggen, Nicholas T. Woods, and Adam R. Karpf
University of Nebraska Medical Center

High-grade serous ovarian carcinoma (HGSOC), the most common and deadliest subtype of ovarian cancer, is characterized by high rates of relapse and resistance, necessitating the development of novel therapeutic approaches. Activation of the forkhead box M1 (FOXM1) transcription factor and pathway is the second most common molecular alteration in HGSOC. FOXM1 promotes cell proliferation, migration, invasion, genomic instability, therapy resistance, and stemness in ovarian cancer. We have previously shown that genetic knockdown of FOXM1 in HGSOC cells leads to decreases in clonogenic growth and sensitizes cells to the PARP inhibitor olaparib, implicating FOXM1 as a viable therapeutic target. 1,1-diarylethylene based drugs, also known as NB compounds, were recently identified from a chemical library screen as FOXM1 inhibitors. We have previously shown that two NB compounds, NB-73 and NB-115, promote proteasome-dependent degradation of FOXM1 and disrupt its transcriptional pathway in HGSOC cells. Importantly, both compounds potently, efficaciously, and selectively inhibit cell viability, induce apoptosis, and decrease clonogenic growth of HGSOC cell models. To better understand the mechanism of action of NB-73 and NB-115 in HGSOC, we performed unbiased proteomic profiling of NB compound effects in two different HGSOC cell lines using tandem mass tag (TMT) mass spectrometry. Bioinformatic analysis indicates that NB-73 and NB-115 exhibit a similar mechanism of action, as evidenced by a strong overlap in differentially expressed proteins and correlation in global effects on the proteome. Transcription factor enrichment analysis of significantly downregulated proteins using Enrichr identified FOXM1, in addition to other known oncogenic transcription factors, including YY1, E2F4, and MYC. Gene ontology of significantly downregulated proteins using Enrichr revealed effects on mitochondrial function, the G2-M checkpoint, and mitotic spindle assembly, consistent with the role of FOXM1 in G2/M and mitotic progression. Conversely, gene ontology of significantly upregulated proteins shows enrichment for processes including apoptosis and the unfolded protein response. We are currently investigating the significance of these pathways with the goal of designing combination treatments to further enhance the efficacy of NB compound FOXM1 inhibitors in HGSOC.

Silencing PAX8 in High-Grade Serous Cancer: A Potential Strategy to Halt Tumor Progression

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This study investigates the critical role of PAX8 in the survival and progression of high-grade serous cancer (HGSC). PAX8 is a master transcription factor that is essential during embryogenesis and promotes neoplastic growth. It is expressed in 90% of HGSC cases, regardless of the cell of origin. PAX8 has previously been shown to regulate HGSC gene expression at both genetic and epigenetic levels, driving cancer cell proliferation, migration, invasion, and tumor formation. In this study, PAX8 loss was found to be associated with decreased secretion of TGF-β, a cytokine crucial for remodeling the tumor microenvironment, as well as the downregulation of TGF-β downstream targets, including Smurf2 and fibronectin. Additionally, PAX8 depletion alters the expression of angiogenesis-related factors, resulting in the downregulation of angiogenic inducers and the upregulation of inhibitors, such as SERPINF1, which has antiangiogenic effects. In vivo, syngeneic mouse models demonstrated significant delays in tumor development and progression in the PAX8 knockdown group compared to controls. Further analysis revealed substantial changes in the 3D fibronectin network and increased immune cell infiltration in PAX8-knockdown tumors. Overall, these findings highlight the crucial role of PAX8 in HGSC progression and the tumor microenvironment, suggesting that targeting PAX8 could be a novel therapeutic strategy for halting HGSC progression.

Community-Based Social Determinants of Health and Ovarian Cancer Survival Disparities

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Social determinants of health (SDOH) are associated with cancer patient outcome, affecting screening rates, diagnosis staging, and complication likelihood. The Social Vulnerability Index (SVI) uses census data to quantify area-based SDOH through four subdomains: socioeconomic status, household characteristics, racial & ethnic minority status, and housing type & transportation. While the SVI has been associated with several health outcomes, no studies have examined the relationship between SVI score and ovarian cancer (OC). We hypothesized that women living at diagnosis in communities with high social vulnerability, defined as within the top quartile of SVI score among Pennsylvania census tracts, would experience poorer survival. Data were extracted from a cohort of 4970 OC patients seen at UPMC in 2000 – 2023. Time-varying adjusted Cox regression models were used to investigate the relationship between SVI and OC survival.

Relative to patients living in communities with low social vulnerability, OC patients residing in communities with high social vulnerability were more likely to have residual disease following surgery (P=0.006) and be diagnosed at an advanced stage (P=0.004). After controlling for relevant demographic and clinical factors, high community social vulnerability was associated with worse survival (adj-HR:1.12[95% CI:1.01-1.24]; P=0.034). Similar associations were found in each of the SDOH domains: race & ethnicity (adj-HR:1.21[1.07-1.37]; P=0.002), household characteristics (adj-HR:1.10[1.01-1.21]; P=0.035), housing type & transportation (adj-HR:1.08[1.00-1.18]; P=0.064), and socioeconomic status (adj-HR:1.09[0.98-1.21]; P=0.118).

Living in areas of high social vulnerability is associated with reduced OC survival. Increasing outreach and available resources to highly vulnerable communities could diminish OC outcome disparities and improve survival.

A Multi-Biomarker Panel for Ovarian Cancer Screening

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Background: Ovarian cancer (OC) is a deadly disease, with the third highest mortality to incidence ratio of all cancers. OC's asymptomatic nature causes identification at an advanced stage in over 70% of patients, at which point the 5-year survival rate is 20%. Differentiating between OC and non-malignant tumors is critical in the clinical setting, especially considering approximately 20% of women will develop an ovarian cyst or pelvic mass in their lifetime and may undergo unnecessary surgery to diagnose it. Despite the need for a predictor for malignant and benign masses, no single biomarker has been able to detect early OC with high sensitivity and specificity; thus there is not an approved biomarker for diagnosis of OC. While cancer antigen 125 (CA125) has been used as a serum marker to monitor patients pre- and post-operatively, CA125 sensitivity is limited in the detection of early OC, and other benign ovarian or non-gynecological conditions have also shown elevated CA125. Therefore, there is a critical need for the development of sensitive and specific biomarkers that allow for early diagnosis of OC in order to identify individuals at high risk for the presence of a malignancy.

Methods: Plasma samples were obtained from a total of 287 women, including individuals with OC at various stages, benign gynecological conditions, and other tumor types as well as healthy non-cancer controls. Samples were analyzed using a curated panel of biomarkers including CA125, Growth Differentiation Factor 15 (GDF15), Human Epididymis Protein 4 (HE4), Hepatocyte Growth Factor (HGF), Leukemia Inhibitory Factor (LIF), Interleukin-6 (IL-6), Osteopontin (OPN), and Prolactin (PRL). Receiver-operating characteristic (ROC) analysis was performed to assess the sensitivity and specificity of single and multiple biomarkers in the various subgroups.

Results/Conclusion: Univariate ROC analysis identified HE4 and GDF15 as having the highest area under the curve (AUC) (AUC=0.8675 and 0.85, respectively) when comparing all stages of OC to healthy non-cancer controls, a value higher than that of CA125 (AUC=0.7621). Multivariate analysis identified the panel containing CA125, GDF15, HE4, IL-6, OPN, and PRL as having the highest AUC when comparing all stages of OC to controls (AUC=0.956) and to benign gynecological conditions (AUC=0.826). This same model also had high specificity and sensitivity when differentiating between early OC and controls (AUC = 0.896). In conclusion, these results validate a novel biomarker panel for early detection of OC and for the distinction of benign versus malignant ovarian masses, including biomarkers not previously studied in OC. Our results are expected to have an important positive impact by accurately detecting premalignant changes or early-stage OC in asymptomatic women.

Epithelial abnormalities in the high-risk fallopian tube of a rare TP53/BRCA2 Li-Fraumeni syndrome patient with multiple tumors

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Background: Li–Fraumeni syndrome (LFS), an inherited familial cancer predisposition syndrome, results from germline TP53 gene mutations thus prompting early-onset sarcomas and carcinomas. Germline double heterozygosity, involving TP53 and BRCA2 mutations (gTP53/BRCA2-DH), is exceptionally rare in LFS patients. Germline mutation of BRCA2 further predisposes affected LFS patients to breast and ovarian cancers as it is associated with Hereditary Breast and Ovarian Cancer syndrome. The combination of these germline mutations, both associated with familial cancer predisposition syndromes, creates a unique cellular and molecular environment within patient tissues characterized by critical deficiencies in mechanisms of genomic maintenance. Furthermore, TP53 and BRCA2 mutations are linked to the stepwise development of fallopian tube (FT)-originating ovarian cancer, though the precise early developmental and molecular changes contributing to carcinogenesis remain elusive.

Methods: A 22-year-old patient with multiple tumors and gTP53/BRCA2-DH presented at Mayo Clinic and participated in a FT biobanking protocol. Immediately post-salpingectomy, tissue was obtained through Tao-brushing of the FT lumen. The collected tissue was subjected to single-cell isolation and single-cell RNA sequencing (scRNA-Seq) for comparison with publicly available normal FT scRNA-Seq data. Validation of lineage markers and aberrantly expressed genes, identified via scRNA-Seq, was performed using highly quantitative droplet PCR assays and immunohistochemistry, respectively.

Results: We generated 1,112 gTP53/BRCA2-DH FT single-cell transcriptomes, including 675 epithelial cells, and compared them with 34,944 public FT single-cell transcriptomes from 3 patients, which included 12,175 epithelial cells. The scRNA-Seq analysis revealed a lack of overlapping clustering within the epithelial populations, suggestive of developmentally altered cell states in the gTP53/BRCA2-DH patient. Specifically, the epithelial cells in the gTP53/BRCA2-DH patient exhibited a substantially altered ratio of multi-ciliated to secretory lineage cells, indicating abnormal differentiation patterns and biases. Differential gene expression and gene set enrichment analysis identified genes and pathways associated with epithelial development downregulated in the gTP53/BRCA2-DH patient and exposed increased inflammatory marker expression along with activation of anti-apoptotic pathways. H&E and immunohistochemical-staining of the FT revealed unique differences in epithelial cell morphological characteristics including an abnormal nuclear-to-cytoplasmic ratio and nuclear shape. Additionally, there was poor distribution and low numbers of multi-ciliated cells throughout the epithelium. Furthermore, there was strong PAX8 expression, high estrogen receptor expression and nearly absent progesterone receptor expression.

Conclusions: This study provided unprecedented insights into preneoplastic changes in epithelial cell development in a rare gTP53/BRCA2-DH LFS patient, shedding light on potential mechanisms underlying cancer development in the FT. Notable changes include alterations in epithelial morphology, bi-lineage differentiation, and molecular state, likely heightening risk for cancer initiation.

Characterizing GAGE2A as a Therapeutic Target in Small Cell Lung Cancer

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Small cell lung cancer (SCLC), despite typically showing an excellent response to the current standard of care chemotherapy, is a very deadly disease. The 7% 2-year survival rate for SCLC is largely due to frequent disease relapse and subsequent chemoresistance. Our lab has previously demonstrated that several cancer testis antigens (CTAs), genes expressed in the placenta and developing testis and re-activated in certain malignancies, are upregulated in a subpopulation of chemoresistant SCLC tumor cells and could be driving the survival advantage for chemoresistant cells. CTAs are understood to be highly antigenic and attractive targets for future therapeutics, but their individual molecular mechanism of upregulation and function in the context of cancer remains broadly unknown. One CTA upregulated in chemo-relapsed SCLC, GAGE2A, has been extensively characterized in this study to assess the subcellular location of the antigen and comprehend the molecular mechanism of GAGE2A upregulation. Four SCLC cell lines were treated with titrated doses of Cisplatin or Etoposide, and GAGE2A expression was measured using immunofluorescence and flow cytometry. We show that robust GAGE2A expression is induced by high-dose chemotherapy. Our data strongly suggest that GAGE2A is located on the extracellular membrane of chemo-relapsed SCLC, making it an amenable and specific therapeutic target. This study provides a first-ever look into the subcellular location of GAGE2A in an attempt to assess its potential therapeutic utility in the future for prevention of chemoresistance.

Characterizing the Tandem Repeat Region of Ovarian Cancer Biomarker CA125

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Current ovarian cancer monitoring relies on an FDA-approved immunoassay called the CA125II test, which detects CA125, an epitope on the large mucin MUC16. However, the CA125II test can only track patient response to treatment and recurrence and is not suitable for population-wide screening. Efforts to make the CA125 test more informative are constrained by the limited understanding of the structure of the CA125 epitopes and MUC16 more broadly. MUC16 contains a tandem repeat region that has been predicted to contain many (up to 63) similar repeats that contain the CA125 epitopes. The highly repetitive nature of MUC16 has historically made it a challenging subject for sequencing and epitope mapping.

In this study, we sequenced the CA125 tandem repeat region by long read sequencing using the Oxford nanopore platform. We sequenced the CA125 tandem repeat region in three ovarian cancer cell lines and in tumor tissue collected from three patients with ovarian cancer. The consensus sequence determined by long-read sequencing was validated with bottom-up proteomics.

Our sequence results reveal that the CA125 tandem repeat region in MUC16 is shorter than previously thought but longer than some mRNA scripts reported in the NCBI database. We verified the sequence we propose is actively translated by identifying unique peptides using LC-MS/MS. Thus, we propose a new CA125 (MUC16) model that contains only 19 tandem repeats rather than 63. Moreover, we identify potential MUC16 splice variants in ovarian cancer patient tissue. The sample contains multiple MUC16 mRNA with different numbers of tandem repeats.

To better understand the binding epitopes of clinically used anti-CA125 antibodies, we predicted the 3D structure of our new MUC16 model with AlphaFold. The predicted structure displays an SEA5 domain and unstructured linker region rich in proline, serine, and threonine residues in all 19 tandem repeats. These results will help us better understand the ovarian biomarker CA125 and will be used for new affinity assay development.

Brigatinib induces synergy with PARP inhibitors through dual inhibition of FAK and EPHA2 in high-grade serous ovarian carcinoma

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PARP inhibitors (PARPis) have now become a standard of care for high-grade serous ovarian cancer (HGSOC), especially for those with defects in homologous recombination (HR) DNA repair. However, the innate and acquired PARPi resistance in HGSOC poses a significant concern, necessitating the exploration of novel treatment options and strategies. Here we investigated the potential of combining brigatinib —a second-generation anaplastic lymphoma kinase (ALK) inhibitor approved for treating lung cancer—with PARPis as way to enhance PARPi activity in HGSOC.

Clonogenic cell survival assays revealed that brigatinib synergizes with PARPis in HR-proficient and -deficient HGSOC cell lines. In subsequent proteomics approaches and cell-based confirmatory studies, we found that brigatinib functions via an off-target mechanism, suppressing the tyrosine kinases FAK and EPHA2. Pharmacologic and gene-silencing techniques demonstrated that dual FAK and EPHA2 inhibition additively reduces downstream PI3K/Akt and MAPK/ERK cell survival signaling, rapidly inducing reduction in levels of FRA-1 which sensitizes cells to PARPi-induced apoptosis. Additionally, the efficacy of this therapeutic approach on tumor size reduction was assessed in HGSOC patient-derived xenograft (PDX) models, with combination treatment inducing tumor regression more effectively than either agent alone. Overall, our studies unveil a beneficial ALK-independent effect of brigatinib that may offer new avenues for considering its repurposing as a HGSOC therapy.

Elucidating How DNA Methyltransferase Inhibition Prevents Platinum-Induced Ovarian Cancer Stem Cell Enrichment

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High-grade serous ovarian cancer (HGSOC) is the most common subtype of ovarian cancer (OC). The current standard of care for OC patients is surgery debulking, followed by a combination of platinum- and taxane-based chemotherapies. Despite the high initial response rate to chemotherapies, most patients experience relapse, and the recurrent tumors no longer respond to platinum and other therapies. Relapse and chemoresistance contribute to a low five-year survival rate of less than 30%, making OC the most fatal gynecological cancer. A population of OC cells termed ovarian cancer stem cells (OCSCs) are enriched in recurrent tumors and drive platinum resistance. In addition to OCSCs, recurrent OC tumors also have aberrant promoter DNA hypermethylation, resulting in the silencing of genes involved in tumor suppression and DNA repair, thus allowing OC cells to survive therapeutic assaults. Our lab has shown that pre-treating OC cells with a DNA methyltransferase inhibitor (DNMTi) blocked platinum-induced OCSC enrichment; however, the mechanism behind this observation is still unknown and appears not to be dependent on altering promoter DNA methylation of tumor suppressor genes. RNA-Seq analysis of OC cells treated with platinum with or without DNMTi pre-treatment identified NF-kB and STAT3 as potential signaling pathways for further examination. NF-kB and STAT3 are transcription factors with known roles in different cellular processes, including stem cell regulation and cancer. Additionally, NF-kB and STAT3 can work together to regulate gene expression, and activation of STAT3 has been shown to influence NF-kB binding to target genes. Our preliminary data showed that in response to platinum and/or DNMTi, NF-kB and STAT3 are activated differently, and NF-kB and STAT3 target genes are differentially expressed. Together, I hypothesize that the combination of DNMTi and platinum activates NF-kB but not STAT3, changing NF-kB genomic binding and leading to changes in transcription of target genes, thus blocking platinum-induced OCSCs. By elucidating how DNA methyltransferase inhibition blocks platinum-induced OCSC enrichment, this study may provide evidence supporting the use of DNMTi combined with platinum as a new frontline therapeutic strategy for OC.

Characterizing the Epigenetic Regulation of Cancer Stem Cell States in High Grade Serous Ovarian Carcinoma

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Therapeutic resistance is a common and critical challenge in the treatment of high-grade serous ovarian cancer (HGSC), as it drives frequent tumor recurrence and significantly contributes to the disease's lethality. In many cancers, including HGSC, cancer stem cells (CSCs) are implicated in treatment resistance, tumor recurrence, and tumor propagation, making them a promising target for therapeutic intervention in HGSC. However, CSCs remain poorly characterized, and effective therapeutic targets are limited. Our unpublished preliminary multi-omic data has identified a chemo-resistant signature associated with known CSC markers, including SOX2. Within our bulk-ATAC seq of representative patient derived xenograft (PDX) tumors and corresponding circulating tumor cells, we found a similar trend where SOX2 was highly enriched within the treatment resistant population when compared to treatment sensitive populations.

When looking into this CSC signature within patient samples, strikingly tissue microarrays showed a significant correlation of SOX2 expression with decreased survival rates. SOX2 is not only implicated in the solid tumor cancer stem cell state, but also has shown strong ties to the regulation of epithelial-to-mesenchymal (EMT) in the metastatic process of circulating tumor cells (CTC), implicating both cell populations as possible CSC states regulating treatment resistance and tumor recurrence. We hypothesize that SOX2 is the identifying factor for CSC states within HGSC responsible for treatment resistance.

Using constitutively over-expressed SOX2 in low endogenous expressing ovarian cancer cell lines and knocked-down SOX2 in high endogenous expressing cell lines, we correlated a loss of SOX2 with improved chemotherapy response. To further characterize the role of SOX2 in treatment resistance, we utilized a doxycycline-inducible lentiviral system to modulate SOX2 expression across multiple HRP in vitro models. We assessed the epigenetic regulation of SOX2 on CSC states and explored the therapeutic resistance characteristics. Further investigation into the role of SOX2 and its potential co-drivers may elucidate the mechanisms of treatment resistance and role on defining cancer stem cells states both in tumors and in circulating tumor cells. Ultimately, we aim to further elucidate the epigenetic regulation behind cancer stem cell states in HGSC and their role in metastasis and therapeutic resistance.

Natural Marine-Derived Fucosylated and Sulfated Glycans Inhibit Notch Activation in Ovarian Cancer Cells

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Ovarian cancer continues to have unacceptably high mortality rates. The identification of methods to intercept disease progression and recurrence are needed. Glycosylated compounds are very safe and stable as drugs, and their medical applications in blocking clotting as well as viral and bacterial pathogenesis have been successful. Prior research indicates that glycan changes, bisecting N-linked and O-fucose, that occur on glycoproteins in ovarian cancer play roles in promoting the expansion of the cancer stem cells or tumor initiating cells. The Notch signaling pathway plays a prominent role in tumorigenesis and is a high priority target for the development of new therapeutics that can block cancer stem cells. In this pilot study we show that the fucosylation and the sulfation groups on certain natural marine-derived glycans are key structural requirements for the inhibition of Notch activation. We measure the binding affinities of these natural glycans with key glycosaminoglycan-binding proteins of ovarian cancer cells such as Wnt using surface plasmon resonance. Using reporter assays for Notch, Wnt, and Hh we determine the efficacy of these natural glycans for single and multiple pathway inhibition. Finally, we demonstrate that the Notch inhibition mediated by natural marine-derived glycans works in synergy with suppression of Notch glycosylation in Radical Fringe knockout cells.

Transcriptional Regulation of Stemness in Clear Cell Ovarian Cancer

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Ovarian Clear Cell Carcinoma (OCCC) represents a formidable challenge in oncology, notorious for its resistance to chemotherapy. Resistance is in part attributed to cancer stem cells (CSCs). While transcription factors (TFs) are recognized as pivotal in shaping the identity of both normal stem cells and CSCs, their role in OCCC chemoresistance and metastasis remains an uncharted frontier. The TF ARID3B is a key regulator of stem cell pathways including Wnt and Myc. ARID3B is emerging as a critical player in cancer by driving CSC identity and abundance. Our groundbreaking research reveals that ARID3B is highly expressed in the nuclei of OCCC patient specimens, Of note, ARID3B's expression correlates with poor prognosis across multiple cancer types including serous ovarian cancer. In xenograft tumors, ARID3B promotes neuroblastoma, ovarian, and head and neck cancer growth, yet its regulation remains a mystery. We discovered that ARID3B is phosphorylated at serine 89. By engineering mutations at this site, we uncovered that phosphorylation status profoundly influences subcellular localization of ARID3B and activation of stem cell-related genes and OCCC xenograft tumor growth. Finally, a preliminary screen identified CAMKK1 as a potential kinase responsible for this critical phosphorylation. Our findings unveil a novel mechanism by which ARID3B drives OCCC progression, opening up innovative avenues for targeted therapy and shedding new light on the molecular underpinnings of cancer stem cell-driven chemoresistance.

Reprogramming of Normal Fibroblasts into Ovarian Cancer-Associated Fibroblasts Via Non-Vesicular Paracrine Signaling Induces an Activated Fibroblast Phenotype

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Introduction: Ovarian cancer (OC) is one of the deadliest forms of cancer in women, where more than 80% of patients develop chemotherapy resistance. Cancer-associated fibroblasts (CAFs) are key contributors to tumor progression and therapeutic resistance through dysregulation of the tumor extracellular matrix (ECM) composition and structure. CAFs may be derived from several cell types through activation and reprogramming by cancer cells, recruitment or epithelial to mesenchymal transition. Thus, CAFs are considered to be notable players in promoting many aspects of tumor function and a promising targeted therapeutic option. While primary CAFs can be isolated from tumors and cultured in vitro, their lifespan is finite with limited expansion capacity, making it difficult to conduct reproducible studies of their role in OC chemoresistance. Therefore, there is a need for translational based approaches that allow for the reprogramming of normal fibroblasts into CAFs. In the absence of reproducible and functionally characterized protocols to engineer and reprogram CAFs in culture, the development of effective anticancer therapies that mitigate CAF-mediated chemotherapy resistance in OC will likely remain difficult.

Materials & Methods: Human primary uterine fibroblasts (HUFs) and immortalized normal ovarian fibroblasts (NOF151-hTERT, NOFs) were reprogrammed into CAFs using tumor-derived conditioned media from two OC cell lines (KURAMOCHI and SKOV-3). HUFs, NOFs, and uterine and ovarian-derived conditioned CAFs (uCAFs and oCAFs) were phenotypically characterized by analyzing fibroblast, CAF, epithelial, and immune makers, as well as their cytokine secretion. Additionally, both conditioned CAFs were functionally characterized by collagen-contraction assay, ALDH activity, promotion of tumor growth (in 3D), and enhancement of drug resistance (in 3D) in comparison to HUFs, NOFs, and primary CAFs. ECM signatures of the uCAFs were assessed through transcriptomic analysis. The activation of CAFs via non-vesicular paracrine signaling was also investigated.

Results/Discussion: Regrettably, we found cell surface marker expression unreliable and just an indicator of activation through culture and exposure to serum as normal fibroblasts were positive for the same stromal and CAF-like markers than the conditioned CAFs. Therefore, we relied upon functional assays to more accurately confirm that the conditioned CAFs display a greater activation status when compared to normal fibroblasts. uCAFs had a significantly higher secretory profile, higher collagen contractility, and higher ALDH activity than HUFs and had similar results to primary OC-CAFs, revealing a clear reprogramming into a CAF-like phenotype. Promotion of cancer growth in 3D cultures revealed that while cancer co-culture with normal fibroblasts does not significantly increase tumor growth neither change drug sensitivity, uCAFs significantly promoted tumor growth and significantly enhanced drug resistance. Moreover, a CAF-like transcriptional signature with involvement of the ECM/matrisome was identified in uCAFs. It was found that non-vesicular paracrine signaling was responsible for fibroblast activation and validated by tumor promotion, ALDH activity, and collagen contractility. The same results were corroborated for NOF and oCAFs. Critically, these results suggest that uterine and ovarian fibroblasts can be reprogrammed into CAFs using tumor-derived conditioned media. In conclusion, our results present a reproducible and functionally characterized protocol to reprogram normal fibroblasts into CAFs using ovarian tumor-derived conditioned media. Moreover, our study is expected to have an important positive impact because it will provide strong evidence for further development of therapeutics that possess potentiality and specificity towards CAF/ECM-mediated chemotherapy resistance in OC and a translatable-based engineering approach that could be applied to different cancer cell types.

Engineering a physiologically relevant in vitro 3D model for precision-based drug screening to study high grade serous ovarian carcinoma

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Ovarian cancer (OC) is often referred to as the "silent killer" due to a lack of symptoms and resistance to treatment that leads to recurrence in 80% of patients within 5 years and is one of the deadliest forms of cancer in women. Lack of efficacy and a low overall success rate of cancer clinical trials are the most common failures when it comes to advancing cancer treatment. Currently available preclinical drug sensitivity screenings present several challenges including an inability to encompass the complexity and heterogeneity of tumors, reproducibility, and a lack of translatability. To overcome these known issues, we have engineered a translationally relevant 100% patient-derived 3D platform with controlled physiologically relevant physical properties to culture tumoroids ("tumor-like-organoids"), which have been shown to retain both histological and genetic features of original tumors and are feasible options for in vitro drug sensitivity assays, to perform clinically relevant assays recapitulating chemotherapeutic responses of patients with ovarian cancer.

Our patient-derived tumoroids (PDT) 3D cultures are developed through the crosslinking of patient-derived plasma fibrinogen to fibrin and patient-derived tumoroids from matched gynecological cancers were embedded within the 3D matrix. This PDT model was fully characterized to show that the model upholds the morphological features, structural complexity and biochemical composition of parental HGSOC tumors. When comparing parental tissue to the PDTs cultured within the 3D model, cytokeratin (epithelial marker, red) revealed the preservation of the native epithelium, tumor microarrays highlighted conservation of tumor heterogeneity, and cytokine arrays validated no changes to important cytokines/signals in the tumor microenvironment. Primary biospecimens were determined to be either sensitive or resistant through the Response Evaluation Criteria in Solid Tumors or RECIST score. PDTs were grown and exposed to standard-of-care carboplatin/paclitaxel treatment for 7 days. To create a predictive score that correlates with the clinical response, several metrics were evaluated including tumor: stroma ratio by QuPath, histopathological screenings for proliferation (Ki67), apoptosis (caspase 3) and CD4/CD8 score, as well as apoptosis screenings by flow cytometry. While a tumor:stroma ratio higher than 50 percent was linked to worse outcomes, histopathological screenings for Ki67, caspase 3 and CD4/CD8 had a moderate prediction. Apoptosis and survival screenings were able to retrospectively match patient response distinguishing sensitive from resistant patients. Critically, these results indicate that this model is a unique preclinical model allowing for the recapitulation of physiologically relevant physical properties which could make it a well-suited platform for precision-based prediction of therapeutic efficacy in gynecological cancers. Our results indicate a reproducible and clinically translatable preclinical model for assessing effective treatment options by predicting therapeutic efficacy and avoiding treatment for patients with drugs that the tumor could have resistance to. Moreover, our results are expected to have an important positive impact because they will provide a valuable tool for establishing a high-throughput drug screening platform that can be used to proactively predict each individual patients' response to therapy to assist in therapeutic selection and permit a much more in-depth and clinically relevant analysis of treatment responses in a precision-based approach than is currently possible.

MUCIN15-Mediated EMT Induction in MUCIN15-Mediated EMT Induction in High Grade Serous Ovarian Cancer Cells Under Fluid Shear StressHigh Grade Serous Ovarian Cancer Cells Under Fluid Shear Stress

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Fluid shear stress (FSS), generated by ascitic fluid dynamics in high-grade serous ovarian cancer (HGSOC), significantly influences tumor progression. Clinically, ascites volume above 1.8L correlates with a 29-month decrease in median survival, underscoring its prognostic importance.

Using computational and experimental approaches, we explored FSS's impact on HGSOC. Patient radiological data and a novel kinematic finite element analysis (FEA) program predicted FSS on the ovarian surface, revealing values up to 11 dynes/cm^2, significantly higher than prior estimates.

We developed a 3D tumor-mimetic model with an agarose-collagen I hydrogel, replicating the ovarian tumor microenvironment (TME). HGSOC cell lines (OVCAR3 and OVSAHO) were exposed to 11 dynes/cm^2 FSS in a bioreactor. Post-exposure, molecular analyses (RNA seq, qPCR, western blot) revealed FSS-induced epithelial-to-mesenchymal transition (EMT), marked by morphological changes, reduced E-cadherin, actin remodeling, and increased invasion/migration capabilities.

Key molecular findings included the activation of p38 MAPK and its downstream effector HSP27, essential for FSS-enhanced motility. FSS also triggered NF-κB nuclear localization, indicative of increased invasion and immunosuppression. The immune microenvironment was altered by heightened secretion of immunomodulatory cytokines, promoting a tumor-supportive macrophage phenotype.

Significantly, FSS downregulated MUC15, a transmembrane glycoprotein, correlating with increased cell motility. MUC15 modulation via knockdown and overexpression confirmed its critical role in FSS responses. G-protein coupled receptors (GPCRs) were implicated as upstream regulators of the p38 MAPK pathway.

Our study highlights the mechanotransduction roles of p38 MAPK and MUC15 in FSS-induced EMT in HGSOC, identifying potential therapeutic targets to impede HGSOC progression and open new avenues for targeted interventions.

Services of the Functional Genomics and Bioinformatics Core

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The Functional Genomics and Bioinformatics Core (FGBC) mission is to assist in molecular biology applications for COBRE project leaders, Sanford Research investigators and regional investigators through project design, resource assessment, sample preparation, assay performance, and data analysis. This Core houses state-of-the-art, high-throughput equipment to enable a wide range of services in genetic and molecular biology analyses, including extraction of nucleic acids from tissues and cells, quantitative and qualitative analysis of nucleic acid samples, gene expression analysis, data analysis and training. During Phase II, more advanced genomics and molecular biology services such as single cell RNA-seq, chIP-seq, RNAscope and assistance with genome editing projects were added. These enhanced services have contributed extensively to publications, successful R01 grant applications, and preclinical studies by Sanford Research investigators. During phase III, the core is beginning to offer more recent and advanced genomics services such as CUT&RUN, CUT&TAG and whole genome CRISPR screens. Additionally, the core is preparing to provide spatial transcriptomics services on the 10X Genomics Visium platform. In addition to services provided in-house, the Core will continue to serve as an interface between investigators at Sanford Research and high throughput sequencing facilities.

Sanford Histology and Imaging Core

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The Histology and Imaging Core (HIC) at Sanford Research was established to support the imaging and histology needs of Sanford Research investigators. The major histology services provided by this Core are processing and embedding tissue, sectioning of paraffin-embedded and frozen tissues, hematoxylin/eosin staining of tissue sections, special staining methods, antibody optimization, immunohistochemistry analysis, and construction of tissue microarrays. The Core maintains a variety of confocal and conventional microscope systems, as well as slide scanning systems for high-throughput imaging. The facility houses three high-resolution laser scanning confocal microscopes (Nikon A1-TIRF, Nikon A1R) that enable investigators to perform confocal and TIRF imaging in live and fixed cells and tissues. The HIC recently acquired a Nikon-CSU-W1-SoRa spinning disk confocal super resolution microscope with four line laser launch and an additional Hamamatsu ORCA FusionBT camera. The Nikon CSU-W1 SoRa uses an innovative optical photo reassignment technique to enable super resolution. The specialized SoRa spinning disk has additional microlenses, combined with the motorized intermediate magnification selector unit it offers additional magnification which brings the XY resolution to 150 nm, subsequent deconvolution of the images can bring the resolution to 120 nm. The super resolution and confocal microscopes are also equipped with climate control chambers with gas mixers to enable live imaging, also under hypoxic conditions. There are two advanced automated microscopes equipped with high-sensitivity monochrome cameras for wide-field fluorescence and color cameras for brightfield capture (Nikon NiE), and a variety of basic microscopes equipped for both fluorescence and brightfield imaging. High throughput equipment includes Aperio Versa slide scanner and Thermo CellInsight CX7 High Content Screening System. The core also maintains several workstations with image analysis packages, including specialized modules for deconvolution.

Optimization of single nuclei preparation for single cell Multiome experiments on ovarian cancer tissue

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Advances in technology in recent years have enabled the study of heterogeneous mixtures of cell populations at the single cell level. scRNA-seq provides whole genome transcription profiling and scATAC-seq identifies accessible chromatin regions at the single cell level. scMultiome technology enables the integrative analysis of scRNA-seq with scATAC-seq to capture multiple layers of information within individual cells. Since the generation of high-quality single-nuclei suspensions is critical for successful scMultiome experiments, particular attention is needed for the optimization of this step for each individual sample. Key elements of "quality" are the integrity of nuclei, the absence of cytoplasm residues around the nuclear membrane and the removal of aggregates. We tested two different tissue digestion methods in ovarian cancer tissue (OC), one based on collagenase digestion and the other using NP-40 as the major detergent, as suggested by the manufacturer (10X Genomics). Collagenase was initially the method of choice since it was previously used in ovarian cancer, however the NP-40 methodology outperformed the collagenase and showed improvements in the quality of nuclei that positively impacted the clustering quality and the downstream analysis of the data. This protocol was optimal also in the processing of frozen tissue, which has the advantage of allowing retrospective sample studies and curated specimen selection with known clinical outcomes. FACS sorting was also tested with no significant differences in sequencing QC results or sample clustering. Visual assessment of nuclei quality was not an accurate enough criterion in the case of tissue embedded in OCT media for cryostat sectioning. In this case test experiment and extraction of RNA from nuclei, followed by assessment of RNA fragment size and integrity was required. We also compared snRNA and scRNA sequencing results for differences in cell identification in PBMC samples and showed that results were consistent, especially considering cell specific markers and abundant transcripts. Our results show how integration of transcription profiling and chromatin accessibility from the same cell, can provide a more complete picture of cell identity and function while maximizing the integrative analysis and the use of precious samples, if the generation of high-quality single-nuclei suspensions is optimal.

A Tri-specific Killer Engager targeting PD-L1 Enhances NK cell immune function against Ovarian Cancer

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Immune checkpoint blockade (ICB) has changed the standard of care for many cancers. Our previous work has shown that maintenance therapy with an IL-15 "superagonist" and ICB PD-L1 in combination could induce potent natural killer (NK) cell activation in ovarian cancer. We made a novel Tri-Specific Killer Engager (TriKER)small molecule containing two different antibody fragments, one against the CD16 activating receptor on NK cells and one against PD-L1 on cancer cells. It also includes an IL-15 linker that drives NK cell survival, priming and expansion. We hypothesize this PD-L1 TriKE will block PD-1/PD-L1 inhibitory signaling and target PD-L1-expressing ovarian cancer cells while providing IL-15 priming and CD16-mediated activation of NK cells. Using flow cytometry we found that PD-L1 TriKE can enhance NK cell functional activity against ovarian cancer cells OVCAR8 after 5hr incubation, by measuring the NK cell degranulation marker CD107a and cytokine produce IFNg. To evaluate the increased NK cell functionality against ovarian cancer cells, we used xCelligence to continuously measure the presence of viable tumor cells over time. This real-time readout showed live OVCAR8s dramatically decreased in the presence of NK cells plus PD-L1 TriKE. Additionally, NK cells combined with the PD-L1 TriKE were shown to significantly improve the tumor growth control in a xenografted mouse model of OVCAR8s. Furtherly, PD-L1 TriKE has also been proofed to enhance NK cell cytotoxicity against PDX derived cancer cells and PDX mouse model survival. These data suggest the PD-L1 specific TriKE can improve NK immune function against ovarian cancer through targeting of the PD-L1 pathways in vitro and in vivo.

Sterol metabolism directly regulates Dishevelled localization and Wnt/beta-catenin signaling

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Wnt/beta-catenin signaling is a critical signaling pathway for cell proliferation, transcriptional activity, and developmental signaling. Aberrant Wnt signaling is broadly associated with a variety of cancer types and cellular processes which promote tumor growth, metastasis, and disease progression. We and others have demonstrated that the Dishevelled (DVL) family of scaffolding proteins, which are necessary for stabilization of the Wnt receptor complex on the plasma membrane, require binding of DVL's PSD95/Dlg1/ZO1 (PDZ) domain to cholesterol to allow Wnt/β-catenin signaling. However, the specificity of this sterol-DVL interaction and the resulting impacts on Wnt and DVL activity has not been determined. Additionally, changes in cholesterol levels and metabolism is frequently associated with tumorigenesis and cancer progression. We hypothesized that changes in membrane-associated sterol biochemistry would functionally regulate DVL activity and Wnt signaling. Protein homology modeling and ligand-protein docking analysis suggested a differential binding affinity and specificity of DVL proteins for cholesterol versus other sterols. Using plasma-membrane mimetic vesicles incorporating cholesterol versus alternative sterols, we observed a reduced binding affinity between DVL2 and alternate sterols in liposome/DVL pulldowns and surface plasmon resonance (SPR) imaging. Mechanistically, molecular simulations and SPR demonstrated that a synergistic interaction between cholesterol and phosphatidylinositol 4,5-bisphosphate (PIP2) lipid species is required to achieve optimal recruitment of DVL proteins to the cytofacial leaflet of the plasma membrane. DVL membrane association was lowest with sterols which poorly support liquid ordered domain formation. Disruption of DVL membrane association by sterols unexpectedly induced significant nuclear accumulation of DVL, including increased DVL protein-protein binding with p53 and other Wnt-independent nuclear proteins. Enhanced nuclear DVL was observed across all cell types tested, including cancer cell lines, immortalized models, neural stem cells, pluripotent stem cells, and disease models of cholesterol synthesis. In summary, our work suggests disrupted sterol homeostasis broadly impacts DVL signaling in a sterol-specific manner with unexpected impacts on cellular signaling. Defining the structural and biochemical requirements for sterol interactions with DVLs and other cholesterol-binding proteins will help define the critical role of sterol homeostasis in development and disease signaling.

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Investigating the Role of Progesterone in the Metastasis of High-Grade Serous Ovarian Cancer

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Ovarian Cancer is the fifth leading cause of death in women and has the highest mortality rate among gynecological cancers, with high grade serous ovarian cancer being the most frequently observed epithelial subtype, which accounts for approximately 70% of ovarian cancer related deaths. Chemical cues or metabolites may help stimulate the migration and colonization of tumorigenic fallopian tube epithelial cells to the ovary, omentum, and other organs. Although, some small molecules may aid in tumorigenesis, other may also play a more protective effect. Through collaboration with the University of California Santa Cruz using Imaging Mass Spectrometry technology, our lab has identified via co-culture of tumorigenic murine oviductal epithelial cells (MOE PTENshRNA) in proximity to a murine ovarian explant unique expression pattern of metabolites, including progesterone. Non-tumorigenic fallopian tube models or models derived from the ovarian surface epithelium did not induce progesterone signaling. Progesterone functions as a steroid hormone involved in female reproductive physiology and studies have shown that can also be used to prevent ovarian cancer and its spread. We used murine oviductal epithelial cells (equivalent of fallopian tube) that endogenously express progesterone receptor (PRA and PRB) and western blots indicated that tumorigenic cells with MOE PTENshRNA had higher PR expression than non-tumorigenic controls. Preliminary data thus far found that with addition of 100nM of synthetic progesterone (R5020) tumorigenic MOE PTENshRNA cell migration is inhibited, but not when these cells express additional tumor drivers such as activated KRAS and mutant p53 (R273H). These studies will help uncover the specific mechanism that progesterone uses to provide this protective ability in high grade serous ovarian cancer (HGSOC).

RHNO1 depletion impairs the DNA replication stress response to strongly inhibit high-grade serous ovarian cancer cell growth

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Background: Genomic instability and defective DNA repair are characteristic of high-grade serous ovarian cancer (HGSOC), which can be exploited as therapeutic targets. We previously reported that Rad9-Hus1-Rad1 interacting nuclear orphan 1 (RHNO1) is overexpressed in HGSOC together with its bidirectional gene partner FOXM1 in HGSOC, contributing to tumor progression. RHNO1 is reported to play a role in ATR/Chk1 signaling and may regulate DNA polymerase theta (POLQ)-mediated end joining (TMEJ), both of which participate in DNA replication stress response and DNA damage repair. However, the precise functions of these processes mediated by RHNO1 in HGSOC are still unknown. In the present study, we investigated the effect of RHNO1 depletion in the replication stress response and DNA repair in HGSOC cells, which is relevant for evaluating the potential of RHNO1 as therapeutic target for HGSOC.

Methods: The effect of RHNO1 depletion on cell proliferation was determined using the Alamar Blue cell viability and colony formation assays in RHNO1 knockdown human and mouse HGSOC cell lines. The DNA replication stress response was evaluated by measuring phosphorylated Chk1 levels following hydroxyurea (HU) treatment. Micronuclei formation, indicative of prolonged DNA damage, was assessed by immunofluorescence. We evaluated the role of RHNO1 in TMEJ using treatments with the POLQ inhibitor ART558 and by measuring RHNO1 association with POLQ by co-immunoprecipitation. We are currently conducting rescue experiments using RHNO1 mutants defective in the replication stress response or POLQ binding to determine the role of these functions in HGSOC cell viability.

Results: In comparison to controls, depleted RHNO1 HGSOC cell lines showed a significantly lower proliferation rate and clonogenicity. RHNO1 knockdown significantly sensitized HGSOC cell lines to HU, leading to increased cell death and micronuclei formation. In contrast, RHNO1 depleted cells showed equal sensitivity to the DNA double-strand break agent etoposide to control cells. RHNO1 knockdown cells exhibited reduced phosphorylated Chk1 levels in response to HU, indicating an impaired replication stress response. Re-expression of wild-type RHNO1 and to a lesser degree SWV-mutated RHNO1 significantly reduced HU cytotoxicity in HGSOC cell lines. RHNO1-deficient HGSOC cell lines showed a slight increase in sensitivity to the POLQ inhibitor ART558, and co-immunoprecipitation demonstrated a direct interaction between RHNO1 and POLQ, which was independent of RHNO1's ATR/Chk1 activating function.

Conclusion: We observed that RHNO1 depletion impairs the DNA replication stress response and strongly inhibits HGSOC cell viability and clonogenic growth. This impaired DNA replication stress response results in increased cytotoxicity by DNA replication stress-inducing small molecules. These results suggest that RHNO1 is crucial for maintaining genomic stability in HGSOC, although its role in POLQ-mediated TMEJ DNA damage repair in HGSOC cells remains to be resolved. By exploiting the vulnerability of HGSOC to agents that induce DNA replication stress, targeting RHNO1 in combination with current standard treatments may improve treatment outcomes for HGSOC.

Targeting Chemotherapy-Induced Toxicity with Macromolecular Superoxide Dismutase Mimetics in a Triple Negative Breast Cancer Mouse Model

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Background: Chemotherapy-induced oxidative stress is a key contributor to both neurological damage and the detrimental side effects of cancer treatments, particularly in patients with triple negative breast cancer (TNBC). A significant number of these patients experience ""chemo brain,"" a condition characterized by cognitive impairment during and after chemotherapy. This study explores the use of two macromolecular superoxide dismutase (SOD) mimetics as a strategy to alleviate oxidative stress and its associated adverse effects, including tumor growth, metastasis, and neurotoxicity, in a TNBC mouse model.

Methods: The therapeutic potential of polynitroxylated albumin (PNA; VACNO) and polynitroxylated pegylated hemoglobin (PNPH; SanFlow) was assessed in 4T1 tumor-bearing mice subjected to chemotherapy. Mice were divided into groups receiving chemotherapy alone or in combination with either VACNO or SanFlow. Tumor progression and metastasis were measured, and brain tissues were analyzed for indicators of neuronal damage and inflammation. Cognitive performance was evaluated using the Ugo Basile Passive Avoidance Apparatus to determine the impact of treatments on behavioral outcomes.

Results: SanFlow combined with chemotherapy led to a significant decrease in both tumor volume and weight compared to the chemotherapy control group. In addition, VACNO and SanFlow both demonstrated neuroprotective effects, as evidenced by decreased Fluoro-Jade C staining, indicating less neuronal damage in brain tissue. Reduced levels of neuroinflammation, as shown by lower CD68 expression, were also observed in SOD mimetic-treated mice. Cognitive testing revealed that mice receiving chemotherapy exhibited cognitive deficits, while those treated with VACNO or SanFlow showed improvements in these parameters.

Conclusion: The results indicate that extracellular SOD mimetics, especially SanFlow, can effectively reduce chemotherapy-induced tumor growth and neurotoxic effects in a TNBC murine model. Ongoing research aims to further elucidate the long-term effects of these agents on neuronal health and cognitive function. These findings highlight the potential of SOD mimetics as a therapeutic approach to mitigating the side effects of chemotherapy in breast cancer patients, improving both oncological and neurological outcomes.

Development of Microfluidic cancer cultures to study Mechanisms of Therapeutic Resistance in High Grade Serous Ovarian Cancer

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A key barrier to understanding how High-grade serous ovarian carcinoma (HGSC) malignant epithelial cells interact with the surrounding tumor microenvironment (TME) in response to therapy is the lack of clinically relevant models. To model all the different cell types in the TME and their interactions in long-term culture, we have developed a microfluidic (μFD) tissue explant platform. Explant μFDs are rapidly established, require only a small amount of tissue, and have improved viability due to concentration of the secretome in the culture chamber and oxygenation through gas-permeable silicone material comprising the devices. Preliminary observations have identified explant growth over time, retention of vasculature, and the benefits of μFDs over standard plate cultures in relation to cell viability and maintenance of tissue architecture. Analysis of cell-specific markers via immunofluorescence (IF) staining revealed that tumor, stromal, and immune cell populations are maintained in culture and resemble that of fresh tissue. Subsequently, we plan to treat μFD cultures with HGSC therapeutic regimens and identify resistance mechanisms from gene expression changes and spatial location of cells within tissue sections using spatial transcriptomics.

Cholesterol metabolism functionally regulates extracellular vesicle transport and release through autophagic induction

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Extracellular vesicles (EVs) are important signaling molecules which transport specific cargo, including nucleic acids, proteins, carbohydrates, and lipids, to recipient cells and tissues. Small EVs, termed exosomes or sEVs, are formed intracellularly through invaginations of late endosomes to produce multivesicular bodies (MVB) prior to extracellular release. sEVs play important roles in cancer pathology, regulating metastasis, angiogenesis, nerve outgrowth, and chemotherapy resistance. As cholesterol is a major lipid component of most mammalian membranes, we hypothesized disruption of cholesterol biosynthesis would affect EV dynamics. Through use of small molecule inhibitors of cholesterol production, we observed that reducing cellular cholesterol increased sEV release. Further, cholesterol levels were negatively correlated with sEV release. Transmission electron microscopy (TEM) of sEVs from cholesterol depleted cells revealed larger and misshapen sEVs relative to controls, and gas chromatography-mass spectrometry analyses showed a severe sterol reduction in sEVs derived from cholesterol-depleted conditions. Using a flow cytometry-based fluorescent bead assay, we also found sEV expression of surface receptors was altered with cholesterol reduction, suggesting potential impacts on uptake of sEVs by recipient cells. Through genetic labeling of CD63- and CD9-expressing sEVs with fluorescent markers, we observed increased uptake of sEVs produced from cells exhibiting impaired cholesterol production. To understand cholesterol impacts on sEV production machinery, we analyzed endosomal sorting by confocal imaging. We found that cholesterol depletion increased expression of Rab5- (early), Rab7- (late), and CD63-positive endosomes, but not Rab11-recycling endosomes. Using TEM, we observed large multilamellar, MVB-like structures in cells treated with cholesterol inhibitors. Additionally, we saw increased LC3B-positive autophagosomes colocalized with Rab7- and CD63-positive endosomes upon cholesterol depletion. We thus hypothesized increased autophagy upon cholesterol inhibition was driving altered sEV biology upon cholesterol depletion. Through intracellular analyses of autophagic flux, we found impaired fusion of autophagosomes with lysosomes after disruption of cholesterol biosynthesis, with autophagosomes being redirected to late endosome-associated structures prior to extracellular release. Utilizing an ATG2A/B CRISPR-deleted cell model to inhibit autophagy initiation, we found that autophagy was required for increased sEV production following cholesterol disruption. Lastly, we have validated cholesterol impacts on sEV production and biology in multiple cellular models, including HPV+ and HPV- head and neck squamous cell carcinoma (HNSCC) cell lines. Our results demonstrate cholesterol regulates sEV production through autophagic signaling, suggesting cholesterol metabolism and autophagy as targets for regulation of sEV biology in cancer.

The Amy Krouse Rosenthal Foundation: Spearheading Innovative Research for the Early Detection of Ovarian Cancer

Deborah Binder, Betsy Katten
Amy Krouse Rosenthal Foundation

Ovarian cancer is the 6th leading cause of cancer-related deaths in women. Research shows that, if ovarian cancer is found before the cancer has spread outside the ovary, the 5-year survival rate is 92%. However, only 15% of all ovarian cancers are found at this early stage. Because we know that early detection exponentially increases a woman's survival rate, the Amy Krouse Rosenthal (AKR) Foundation focuses on early detection research, education and awareness. Among the Foundation's programs and efforts, we are proud to provide grant funding for groundbreaking ovarian cancer early detection research.

Functional Genomics and Bioinformatics Core Services

Malini Mukherjee, Jessica Roberts, Oduduabasi Isaiah, Benjamin Derenge, Michael Kareta, and Kameswaran Surendran
Sanford Research

The Functional Genomics and Bioinformatics core (FGBC) mission is to assist in molecular biology applications for COBRE project leaders, Sanford Research investigators and regional investigators through project design, resource assessment, sample preparation, assay performance, and data analysis.

Biochemistry Core: Exploring Protein Networks with Cutting Edge Techniques

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Sanford Research

In the post-genome era there is increased emphasis on understanding protein function. A powerful approach to study protein function is to monitor protein-protein interactions (PPIs). Traditional methods to screen for PPIs, including yeast two-hybrid and affinity-protein complex purification, have been successful but have substantial limitations. To overcome some of these limitations and provide a complementary approach, the Biochemistry Core (BC) at Sanford Research primarily functions to provide BioID services. BioID (for proximity-dependent biotin identification) is a fundamentally unique method to generate a history of PPIs over time by using a promiscuous biotin ligase fused to a bait protein or targeting motif. When expressed in cells the BioID fusion protein biotinylates proximate proteins enabling their capture and identification by mass-spectrometry analysis. Unlike other approaches, BioID is applicable to insoluble proteins and enables detection of weak and transient PPIs. Since we developed the method in 2012, BioID has become an established method with over 300+ publications citing or using BioID to monitor PPIs in a wide variety of cellular and animal models. The BC also works to improve the BioID method by advancing the “BioID-Toolbox” by creating BioID2 biotin ligase, expanding zipcode BioID, and testing a recently engineered, faster version of BioID termed TurboID. In addition to BioID services, the BC assists researchers investigate PPIs by providing training, and performing conventional co-immunoprecipitation and gel/membrane imaging.

Flow Cytometry Core Facility

Jared Wollman and Dr. Pilar de la Puente
Sanford Research

The Flow Cytometry Core was established in 2011 through funding provided by the NIH CoBRE Grant – Cancer Biology Research Center. The Core is housed within the Sanford Research Center; employing experienced, technically proficient staff; and maintains, operates, and supports multiple research instruments for flow cytometry, flow sorting, and sample preparation. The Core's primary mission is to fulfill the flow cytometry-based needs of Sanford Research's principal investigators and neighboring institutions.

Towards Off-The Shelf Natural Killer Cell Products For The Treatment Of Ovarian Cancer

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Cell immunotherapy, using Natural Killer cells (NKc), is a promising treatment for ovarian cancer. One way to alleviate variability between expanded NK cells (eNKc) donors is to co-deliver Tri-specific Killer Engager (TriKE) biologic molecules. These molecules direct NKc killing towards a B7H3 target on ovarian cancer cells and increase NKc persistence through the IL-15 moiety present in the TriKE. Here, we examine the impact of eNKc donor variability on cytotoxicity when treated with B7H3 TriKE. Our studies compared the donor efficacy of eNKc donors in combination with B7-H3 TriKE in vitro and in vivo. After 4 hours, xCELLigence data showed treatment of OVCAR-8 cells with eNKc + 0.3 or 3 nM TriKE (67.10±18.61% and 72.17±20.32%) provided superior cytotoxicity to eNKc alone (44.81±15.10%). These trends in cytotoxicity persisted over 12 hours. Based on BLI, Donor 3 showed a significant improvement in the eNKc + TriKE group compared with the control at days 7, 14, and 21 while Donor 4 showed no significant improvement at any time point. We show that eNKc with TriKE is significantly more cytotoxic than eNKc alone in vitro and that this cytotoxic trend persists over time. In vivo, eNKc with TriKE controls tumor burden to day 21, but results were donor-dependent. The correlation between in vitro and in vivo results highlights the importance of testing donor functionality before utilization in animals and ultimately humans.

Veratridine lessens cell migration and spheroid formation in colon cancer in a P53-independent manner

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Colorectal cancer (CRC) causes approximately 900,000 deaths annually worldwide. The survival rate for patients diagnosed with localized CRC is 90 percent; however, once the cancer metastasizes or spreads to other parts of the body, the five-year survival rate drops to a dismal 15%. This shows a clear need for a new generation of drugs that can safely and efficiently target the metastatic form of CRC. Our lab has shown that Veratridine (VTD), a lipid-soluble plant alkaloid, can enhance the expression of a ubiquitin-like protein called UBXN2A as a colon-specific tumor suppressor protein. UBXN2A targets and negatively regulates the Rictor protein, a key component of the mTORC2 signaling pathway. This results in suppression of mTORC2-AKT's downstream signaling pathway in cancer cells. We hypothesize that the VTD-UBXN2A axis will decrease migration in cells and spheroids independent of p53. HCT-116 (WT p53), and HT-29 (mutant p53) cells and their 3D spheroid forms will be used to complete this project. Real-time cell migration and invasion analysis using xCELLigence technology and Cytation 1 cell imaging system will determine whether the VTD-UBXN2A axis can suppress metastatic characters of CRC cells with wild-type (WT) and mutant forms of p53 protein. This ongoing study will provide detailed insight into the therapeutic efficacy of VTD-UBXN2A in two different stages of CRC cells.

Design and Synthesis of FDI-6-Based Hydrophobic Tag Degraders Targeting FOXM1 Degradation

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FOXM1, a forkhead box transcription factor, is overexpressed in numerous human cancers and this is often associated with poor prognosis. FOXM1 regulates transcription by binding to target gene promoters and interacts with key oncogenic pathways. Previously, small molecule inhibitors have been used to target FOXM1 by disrupting DNA binding, with FDI-6 being the first selective inhibitor reported. However, FOXM1 has functions besides DNA binding, indicating the need for a better targeting approach. Targeted protein degradation, including proteolysis-targeting chimeras (PROTACs) and hydrophobic tag (HyT) degraders, is an alternative approach for FOXM1 inhibition. An FDI-6-based PROTAC for FOXM1 has been reported, leading us to hypothesize that an FDI-6-based HyT degrader could also regulate FOXM1 levels. By replacing the cereblon ligand in the PROTAC with a hydrophobic group, we aim to reduce off-target effects and increase specificity for FOXM1. HyT degraders bind to the surface of target proteins, exposing hydrophobic regions that can mimic the characteristics of misfolded proteins, which in turn triggers the protein quality control system to induce proteasomal degradation. To test our hypothesis, we designed and synthesized FDI-6-based HyT degraders using norbornene or adamantane as the hydrophobic group. The HyT degraders were tested against OVCAR4 ovarian cancer cells. A reduction in cell viability was seen with increasing concentrations, suggesting the compounds are effective in this cell line. Additionally, target engagement studies showed a decrease in FOXM1 protein levels, suggesting that HyT degraders may be a promising approach for targeting FOXM1.

Characterization of anti-CA125 Monoclonal Antibodies by Western Blot and ELISA

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University of Kansas

To improve ovarian cancer survival rates, better diagnostic tools must be developed to detect the cancer at earlier stages. The current FDA-approved ovarian cancer surveillance test, the CA125 blood test, monitors levels of CA125 in the blood using a sandwich ELISA (enzyme-linked immunosorbent assay) with the M11 and OC125 antibodies. CA125 is an epitope on MUC16, a heavily glycosylated protein biomarker containing a region of 19 tandem repeats. CA125 is located within the tandem repeat region, but little is known about its exact location, structure, and role in ovarian cancer progression. To gain insight into CA125's structure, we probed 16 of the 19 individual repeats with M11-like and OC125-like monoclonal antibodies, which have been reported to bind to CA125 similarly to the clinically used antibodies. This project aimed to characterize two M11-like clones (M002203 and M77161) and two OC125-like clones (M002201 and X306) using Western blot and indirect ELISA techniques. Western blot was used to detect binding between the antibody and denatured repeat proteins, revealing potential linear epitopes. ELISA was used to detect binding between the antibody and proteins in their native state, examining both linear and conformational epitopes. The two M11-like clones displayed binding to more repeats across the tandem repeat region compared to the two OC125-like clones, which bound to less repeats across this region. The M11-like clones also exhibited different binding patterns while the OC125-like clones showed similar binding patterns on the Western blot and ELISA. Future work will utilize surface plasmon resonance (SPR) as an additional method for characterization of these antibodies. In addition, more antibody-antigen binding interactions may be studied to gain further insight into CA125's location and structure.