Welcome to the 2026 Global Cancer Consortium Conference

POSTER ABSTRACTS

The Hormel Institute

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We are pleased to present this collection of abstracts highlighting the innovative research and collaborative efforts of investigators, clinicians, and trainees from around the world.

We hope this conference provides valuable opportunities for scientific exchange, networking, and the development of new collaborations.

Thank you for joining us, and we wish you a productive and enjoyable conference.

About the Abstract Book

Abstracts are organized first by conference session and then alphabetically by the last name of the presenting author. Accepted abstracts are designated according to presentation type using the following key:

SA = Selected Abstract

SAA = Selected Abstract Alternate

FT = Flash Talk

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Virtual: DNA methylation profiling identifies a convergent, lineage-agnostic brain metastasis subtype with prognostic significance and therapeutic vulnerabilities [SA]

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Abstract

Brain metastases (BrM) represent a frequent and devastating manifestation of systemic cancer. Emerging evidence suggests that BrM possess intrinsic molecular features shaped by adaptation to the brain microenvironment that influence clinical behavior and therapeutic response, yet molecular classification of BrM has lagged considerably behind that of primary brain tumors, in part due to the heterogeneity imposed by disparate primary sites of origin. To address this, we assembled a large, multi-modal cohort of 548 surgically resected brain metastases spanning the most common solid tumor origins. Through DNA-methylation profiling we identified a distinct, lineage-agnostic cluster of BrM, which we term Poly-Origin BrM, defined by convergent molecular signatures that diverge from their primary cancer site of origin. Compared to tumors retaining origin-aligned epigenetic profiles, Poly-Origin BrM demonstrate a significant and independent overall survival advantage (20.2 vs. 10.6 months; HR 0.69, $p = 0.013$). Integrating bulk transcriptomics, methylation deconvolution, and single-cell RNA sequencing, we show that Poly-Origin BrM are characterized by convergent epigenomic reprogramming, reduced mitotic age, and relative genomic stability, and harbor a pro-inflammatory tumor microenvironment enriched for immune-active macrophage states and cytotoxic lymphocytes, alongside less differentiated, developmentally plastic neoplastic programs. Using machine learning, we derived a Poly-Origin methylation signature score that validates robustly across two independent external cohorts. Clinically, Poly-Origin classification identifies patients with striking differential benefit from adjuvant targeted therapy and immunotherapy (48.9 vs. 17.0 months; HR 0.36, $p < 0.001$), with no comparable benefit observed in Origin-Aligned tumors, suggesting that methylation-based subgroup assignment may serve as a predictive biomarker for treatment selection. Finally, the Poly-Origin signature is detectable non-invasively from peripheral plasma using cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq), enabling prognostic stratification without tissue biopsy. Together, these findings establish a convergent, lineage-agnostic BrM subtype with distinct epigenetic, immune, and developmental biology, and provide a framework for biologically informed risk stratification and therapeutic selection.

1. Primary Cilia Loss Drives EZH2-Dependent Epigenetic Reprogramming in Cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a highly lethal cancer arising from the malignant transformation of cholangiocytes. Therapeutic options remain limited, and most patients present with advanced disease, when resection or transplantation is no longer feasible, resulting in a median survival of under 24 months. A defining yet understudied feature of CCA is the near-universal loss of primary cilia. Rather than a passive consequence of transformation, ciliary loss reflects an active deciliation process driven by destabilization of the ciliary axoneme. Experimental deciliation of normal cholangiocytes induces hyperproliferation, anchorage-independent growth, and activation of MAPK and Hedgehog signaling, whereas restoring cilia in CCA cells suppresses these behaviors, although the mechanistic basis remains unclear. Epigenetic deregulation, particularly via the histone methyltransferase EZH2, is a major driver of cholangiocarcinogenesis. EZH2 catalyzes trimethylation of histone H3 on lysine 27 (H3K27me3) to repress cell-cycle inhibitors such as p21, and its overexpression promotes tumor growth and silences ciliogenesis-related genes in other cancers.

Here, we demonstrate that primary cilia loss directly enhances EZH2 activity in cholangiocytes. Deciliated cells exhibit increased EZH2 protein, elevated H3K27me3, and reduced p21. Mechanistically, deciliation requires HDAC6-dependent deacetylation of axonemal tubulin, leading to microtubule destabilization and ciliary disassembly. HDAC6 overexpression increases EZH2 activity, whereas restoring axonemal acetylation through ATAT1 suppresses EZH2 in HDAC6-high tumor cells. Deciliation is further associated with β-catenin activation, and β-catenin knockdown decreases H3K27me3, supporting a cilia–Wnt–EZH2 axis linking ciliary loss to nuclear epigenetic remodeling. In vivo, cholangiocytes from IFT88-deficient mice, which cannot assemble cilia, display markedly elevated H3K27me3, consistent with enhanced EZH2-dependent transcriptional repression.

Together, these findings position primary cilia dysfunction as an upstream regulator of EZH2 in CCA, revealing a previously unrecognized cilia–epigenetics pathway and suggesting that restoring ciliary integrity or targeting EZH2-driven chromatin remodeling may offer new therapeutic strategies.

2. The primary cilium is linked to hepatic stellate cell activation in the liver microenvironment [FT]

Borah, N.A., *Dubravcic, B., Peixoto, E., Pant, K., Kang, N., Gradilone, S.A.*

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Abstract

Background: The hepatic tumor microenvironment is a critical regulator of primary and metastatic liver cancer progression. Hepatic stellate cells (HSCs) are key stromal cells that, upon activation, acquire a cancer-associated fibroblast-like phenotype and promote extracellular matrix remodeling, fibrosis, and tumor-supportive signaling. Although TGFβ is a central driver of HSC activation, the subcellular platforms that regulate this process remain poorly defined. The primary cilium is a sensory organelle that coordinates diverse signaling pathways. We hypothesized that the primary cilium functions as a regulatory hub for TGFβ-mediated HSC activation.

Methods: Primary cilia in HSCs were examined by immunofluorescence microscopy. The localization of TGFβ receptors I and II relative to the ciliary compartment was assessed using co-localization studies. To determine whether the primary cilium is functionally required for HSC activation, ciliogenesis was disrupted by shRNA-mediated knockdown of the ciliary transport protein IFT88. Control and IFT88-knockdown HSCs were stimulated with TGFβ1, and activation was evaluated by immunofluorescence and immunoblotting for α-smooth muscle actin and type I collagen.

Results: HSCs consistently displayed primary cilia. TGFβ receptors I and II localized in close association with the ciliary compartment, particularly near the ciliary base. Importantly, IFT88 knockdown attenuated TGFβ1-induced upregulation of α-smooth muscle actin and type I collagen compared with cilia-preserved control cells. These findings suggest that intact primary cilia are required for efficient TGFβ-mediated activation of HSCs.

Significance: This study identifies the primary cilium as a previously underappreciated regulator of hepatic stromal activation. By linking ciliary signaling to HSC activation, our findings provide new insight into the liver tumor microenvironment and highlight a potential stromal vulnerability. More broadly, these data support the development of therapeutic strategies aimed at disrupting tumor-supportive stromal programs rather than targeting cancer cells alone.

3. The Role of NALCN-regulated cancer associated fibroblast activation in colorectal cancer metastasis to the liver

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Abstract

Metastasis is the leading cause of cancer-related deaths, with liver metastases particularly lethal in colorectal cancer (CRC), reducing 5-year survival to ~30% due to liver failure and limited effective therapies. Successful metastatic colonization of the liver requires dynamic tumor-stroma interactions that enable disseminated CRC cells to evade dormancy and initiate outgrowth. Dr. Rahrmann previously identified the sodium leak channel NALCN (Na^+ leak channel, non-selective) as a key regulator of metastatic progression. NALCN is significantly mutated in human CRC, and its deletion in a mouse model of intestinal adenocarcinoma increases circulating tumor cell release and distant metastases, including the liver. While interrogating CRC liver metastases, we discovered NALCN is expressed not only in epithelial tumor cells but also in cancer-associated fibroblasts (CAFs), suggesting that NALCN has a previously unrecognized role in the tumor microenvironment (TME).

While NALCN regulates epithelial-to-mesenchymal transition, invasion, and metastasis in epithelial cells, its function in fibroblasts and tumor-stroma crosstalk remains unknown. In collaboration with Dr. Kang, an expert in the hepatic TME, and building on our lab's discovery of epithelial NALCN, I identified its previously unrecognized expression in quiescent hepatic stellate cells (HSCs)-precursors of the CAFs in CRC liver metastases. Transforming growth factor beta (TGF-β), secreted by CRC cells and resident liver cells, activates HSCs into metastasis-supporting CAFs. Our preliminary data demonstrate TGF-β1 increases NALCN expression in primary human HSCs and that NALCN is required for their myofibroblast activation. shRNA-mediated knockdown of NALCN prevents HSC alpha-smooth muscle actin (αSMA) expression, a marker of HSC activation, revealing a new link between TGF-β1 signaling and NALCN expression in the hepatic TME. We hypothesize that TGF-β1–induced NALCN expression in HSCs promotes the development of a prometastatic liver microenvironment by regulating fibroblast behavior.

4. Investigating LYVE-1+ Macrophage Efferocytosis in Triple-Negative Breast Cancer [SAA, FT]

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Abstract

Breast cancer is the second most common cause of cancer-related death in women. While targeted therapies for triple-negative breast cancer remain limited, macrophages within the tumor microenvironment (TME) are a promising target due to their role in driving invasion and immunosuppression. Our lab has identified a subset of LYVE-1+ macrophages that localizes to the invasive edge of the tumor, contributes to tumor growth, and promotes both extracellular matrix remodeling and immunosuppression. The clearance of apoptotic cell debris, referred to as efferocytosis, is a key function of macrophages and is associated with immunosuppression and tissue remodeling. In vivo experiments in our lab utilizing a Lyve-1CrexCsf1rfl/fl macrophage depletion mouse model demonstrated increased apoptosis in the tumor margin compared to wild-type mice, suggesting that LYVE-1+ macrophages contribute to clearance of apoptotic debris. LYVE-1+ macrophages also localize with fibroblasts at the tumor margin, suggesting that they could engulf fibroblasts, as well as invading tumor cells. Furthermore, recently published studies by others have demonstrated that different types of apoptotic cells can drive distinct phenotypes in macrophages, potentially impacting their function. The impact of different types of apoptotic debris in the TME on macrophage phenotype has not previously been studied and highlights a novel mechanism by which tumors drive macrophage function. To fill this gap, I have cultured apoptotic cells of different lineages with LYVE-1+ macrophages and performed RNA sequencing to define transcriptional shifts in the macrophages. My work aims to define tumor, TME, and LYVE-1+ macrophage crosstalk to identify new therapeutic axes for breast cancer.

5. Investigating synergistic impact of diet-induced hyperinsulinemia and mutant KRAS on colorectal cancer metastasis [FT]

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Abstract

Purpose: This study examines how western style diet–induced metabolic dysfunction affects cancer metastasis in an in vivo, genetically engineered iKAP model (inducible Kras, Apc, and p53) (Boutin et al., Genes & Development, 2017, 31:370).

Methods: 8-10 weeks old mice are randomized to either a 10% low-fat-diet (healthy diet) or a 45% high-fat-diet (western-style diet) to induce hyperinsulinemia and metabolic dysfunction before tumor initiation. The development of metabolic dysfunction is monitored using Echo-MRI for alterations of body composition, glucose tolerance test (GTT), and endpoint steatosis. Upon tumor induction, tumor growth rates are measured to compare tumor burden in relation to diet and Kras mutation status. Micro- and macro-metastases are recorded and compared using micro-CT imaging and endpoint tissue histopathology. In parallel, a complementary in vitro study using a panel of isogenic colorectal cancer cell lines is underway to evaluate the effect of insulin-mediated cell migration and invasion through scratch and trans-well assays in the presence of mutant KRAS, as well as downstream signaling analyses by western blot.

Result: Our preliminary in vivo findings demonstrate signals of metabolic dysfunctions, including higher body fat percentage, lower glucose tolerance, and the development of hepatic steatosis in HFS-fed iKAP mice. Mice on the 45% high-fat diet showed higher liver disease scores compared to those on the 10% fat diet. Inclusions of hepatocyte ballooning and inflammation were noticeably increased in the high-fat group. Endoscopy shows primary colon tumor formation.

Conclusion: Based on preliminary findings, western style diet effectively induces metabolic dysfunction in the iKAP in vivo model. Additional mice and sex-based analyses will further validate and strengthen these findings. This context may support investigating hyperinsulinemia-oncogenic Kras crosstalk in CRC progression and metastasis. Moving forward, chronic hyperinsulinemia reprograms metabolism and intracellular signaling in the presence of mutant Kras to expand tumor metastatic spread, is an open question.

6. Space: an NK Cell Frontier[FT]

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Abstract

NK cell cytotoxicity assays are a cornerstone experiment used to gain insight regarding the biology of these frontline cancer fighters. However, the results of these assays are highly variable, even under conditions that have been historically treated as analogues. We find that a source of this variability at identical E:T ratios is the density at which experiments are seeded. This highlights the spatially-dependent nature of NK cell killing. Convention has widely believed that the in vitro killing capabilities of NK cells is proportional to their number or E:T ratio. Our findings regarding the spatial dynamics within cytotoxicity assays disproves these beliefs; demonstrating an instance in which fewer NK cells at a lower E:T ratio were responsible for more cytotoxic events than their counterpart. Additionally, the relationship between proximity of NK cells to target cells and percent cytotoxicity required minimal framework to accurately model. The existence of this relationship supports the notion of "scarcity" dictating the likelihood of immune elimination of DTCs and prompts further investigation into how to overcome this significant physiological hurdle.

7. TNFRSF12A Signaling in Hepatic Stellate Cells Promotes a Tumor-Permissive Liver Microenvironment in Colorectal Cancer Metastasis [FT]

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Abstract

Colorectal cancer (CRC) liver metastasis remains a major cause of cancer-related mortality, yet the stromal mechanisms that establish a tumor-permissive hepatic microenvironment are incompletely understood. Hepatic stellate cells (HSCs) are central regulators of liver fibrosis, inflammatory remodeling, and immune signaling during chronic liver injury and tumor progression. However, the contribution of HSC-intrinsic TWEAK-TNFRSF12A/FN14 signaling to metastatic niche formation has not been defined. Here, we investigated the role of TNFRSF12A signaling in activated HSCs during CRC liver metastasis. Our preliminary studies demonstrate that TGF-β robustly induces TNFRSF12A expression in primary human HSCs, accompanied by activation of inflammatory and cytokine-associated pathways. Mechanistically, we identified EGR2 as a candidate transcriptional regulator of TNFRSF12A induction in HSCs. Functionally, activation of the TWEAK-TNFRSF12A axis enhanced expression of tumor-promoting cytokines and macrophage-associated factors, including CCL2, CSF1, and IL-6. Importantly, TWEAK stimulation of HSCs significantly enhanced colorectal cancer cell growth and migration in 3D culture and transwell systems, supporting a paracrine role for HSC-derived signaling in metastatic progression. In contrast, genetic deletion or pharmacologic inhibition of TNFRSF12A using a selective antagonist markedly suppressed these tumor-promoting effects. Furthermore, the TWEAK-TNFRSF12A axis remodels macrophage phenotypes by promoting macrophage polarization and suppressing phagocytic activity within the metastatic liver microenvironment. Collectively, these findings identify HSC-specific TWEAK-TNFRSF12A signaling as a previously unrecognized regulator of the metastatic liver niche and suggest that stromal TNFRSF12A signaling may represent a promising therapeutic target for colorectal cancer liver metastasis.

8. CIN-driven macrophage differentiation confers ICI resistance in NSCLC lymph nodes

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Abstract

Introduction: Immune checkpoint inhibitors (ICIs) combined with chemotherapy have become first-line treatment for advanced non-small cell lung cancer (NSCLC). However, a substantial proportion of patients develop resistance, with lymph node progression representing a dominant failure pattern. The mechanisms underlying lymph node-specific resistance remain poorly understood. We previously identified a unique macrophage subset (Maco_prolif) enriched in resistant lymph nodes that expresses high levels of CXCR3 and exhibits genomic instability. We hypothesized that CXCR3 signaling drives chromosomal instability (CIN) in Maco_prolif cells, leading to formation of immune-privileged germinal centers (GCs) that mediate ICI resistance.

Methods: We performed single-cell RNA sequencing on paired primary tumors and lymph nodes from NSCLC patients receiving chemoimmunotherapy. Multiplex immunofluorescence was used for spatial validation. Mechanistic studies utilized the OPCM ICB-resistant mouse model with LLC1-ICB-R cells. Interventions included CXCR3 inhibitor AMG487, STING inhibitor C-176, and anti-PD-1, alone or in combination. CIN70 scoring, γH2AX immunofluorescence, DNA fiber analysis, and flow cytometry were performed. Statistical analyses used two-sided Wilcoxon test, ANOVA, and log-rank test.

Results: CXCR3$^+$ Maco_prolif cells enriched in resistant lymph nodes exhibit high CIN. scRNA-seq revealed significant enrichment of CXCR3$^+$ proliferating macrophages in resistant lymph nodes (BL) versus sensitive nodes (AL). CIN70 scores were higher in BL Maco_prolif cells, with CXCR3 expression positively correlated with CIN70. mIHC confirmed elevated γH2AX in BL CXCR3$^+$ Maco_prolif cells versus controls.

CXCR3 signaling directly induces replication fork conflicts and CIN. Recombinant CXCL10 stimulation of Maco_prolif cells induced γH2AX foci and p-RPA3 upregulation, abrogated by CXCR3 antibody. DNA fiber analysis confirmed reduced replication fork speed upon CXCR3 stimulation.

CXCR3-induced CIN activates STING-NF-κB-CCL18 axis. Conditioned media from resistant tumor cells activated STING-NF-κB signaling and CCL18 secretion in Maco_prolif cells, blocked by CXCR3 or STING inhibitor. ChIP-qPCR confirmed NF-κB binding to the CCL18 promoter in LAM-CCL18$^+$ cells. mIHC revealed LAM-CCL18$^+$ cells co-localized with GCBs in BL, with CD8$^+$ T cell exclusion.

CXCR3 blockade combined with anti-PD-1 reverses resistance. In the OPCM model, CXCR3 inhibitor plus anti-PD-1 reduced lymph node metastasis and prolonged survival versus monotherapies. Combination therapy disrupted LAM-CCL18$^+$-GCB co-localization, reduced γH2AX, restored CD8$^+$ T cell function, and normalized GCBs.

Conclusion: We demonstrate that CXCR3 signaling in Maco_prolif cells induces CIN, activating the STING-NF-κB-CCL18 axis. This drives differentiation into LAM-CCL18$^+$ macrophages that co-localize with GCBs via CCL18-CCR8, forming immune-privileged germinal centers that exclude CD8$^+$ T cells and promote ICI resistance. Targeting CXCR3 in combination with anti-PD-1 disrupts this ecosystem and reverses resistance, representing a promising therapeutic strategy for NSCLC patients with lymph node progression.

9. Loss of Sodium Leak Channel (NALCN) Impairs Angiogenic Potential in Endothelial Cells SAA, FT

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Abstract

Beyond its established role in maintaining neuronal resting membrane potential, the sodium leak channel (NALCN) has recently been identified as a key mediator of epithelial cell trafficking and metastasis. Using the 4T1 syngeneic triple-negative breast cancer (TNBC) model, we demonstrated that shRNA-mediated Nalcn suppression significantly reduced survival and promoted peritoneal metastases. These data suggest NALCN is a metastasis suppressor in TNBC.

A pivotal shift in our investigation occurred upon observing robust NALCN expressions within the tumor microenvironment (TME). Specifically, NALCN was highly expressed in normal tissue vasculature surrounding the metastatic site. Given that ion channels fuel cell motility and allow tumors to reshape their surroundings for easier spread, this spatial expression pattern suggested that NALCN's influence might extend far beyond the tumor parenchyma.

By defining the bioelectric mechanisms governing endothelial cell (EC) biology, we aim to develop therapeutic strategies that "fortify" the vasculature, effectively sealing pathways used for metastatic escape and improving outcomes for patients with aggressive malignancies. We, therefore, hypothesize that NALCN regulates cell-cell junctions and that its loss destabilizes the vascular barrier, facilitating the "seeding" of circulating tumor cells in the lungs.

To test this, we developed a first-of-its-kind tamoxifen-inducible, endothelial-specific Nalcn knockout mouse model (Pdgfb-iCreER;Nalcnflx/flx). Deletion was confirmed in CD31+ lung-derived ECs. Structural analysis of ZO-1, a tight-junction marker, revealed that Nalcn loss results in shortened ZO-1-positive branches and increased junctional breaks, indicating impaired microscopic barrier integrity. Surprisingly, in vivo assays showed that Nalcn deletion does not alter baseline vascular homeostasis or VEGF-induced permeability. This suggests that NALCN-mediated bioelectric signaling and the VEGF-VEGFR2 axis operate through distinct, non-overlapping pathways. However, functional testing of Nalcn-deficient primary lung ECs revealed a significantly diminished capacity for angiogenic vessel formation, mirroring results from human EC knockdowns and confirming NALCN's necessity for standard endothelial function.

We are currently utilizing this model to determine how an altered vascular niche influences tumor seeding to lungs, ionic conductance in tumor microenvironment, and organotropism. Ultimately, this research positions NALCN as a vital stromal ion channel regulator.

10: Targeting ALKBH5 RNA demethylase Alters m6A Landscape, Myofibroblastic Activation, and Cytokine Secretion of Hepatic Stellate Cells

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Abstract

Introduction: ALKBH5 is an RNA demethylase that removes N6-methyladenosine (m6A) modifications from RNA molecules so as to regulate RNA metabolism, such as splicing, stability, and nucleus-to-cytoplasm export of RNA as well as translation. Hepatic stellate cells (HSCs) are activated into cancer-associated fibroblasts (CAFs) that are important cellular components of colorectal liver metastases. This study explores whether and how m6A modification regulates the function of HSCs as well as myofibroblastic activation of HSCs.

Methods: ALKBH5 of HSCs was knocked down by shRNA lentiviruses. HSC activation was evaluated by Western blot (WB) and immunofluorescence (IF) analysis of HSC activation markers. Bulk-cell RNA sequencing (RNA-seq), m6A RNA immunoprecipitation followed by sequencing (MeRIP-seq), and Bioinformatic analyses were performed to identify differentially expressed genes (DEGs) induced by ALKBH5 knockdown and transcripts undergone m6A modifications. A targeted proteomics was employed to detect cytokine secretion of HSCs.

Results: WB and IF revealed that TGFβ1 stimulation increased HSC expression of α-smooth muscle actin (αSMA), CTGF, Fibronectin, indicating HSC activation; notably, this effect of TGFβ1 was markedly suppressed by knocking down ALKBH5 of HSCs ($P < .05$). RNA-seq revealed that knocking down ALKBH5 led to global changes in the TGFβ-responsive transcriptome, including downregulation of 2371 transcripts and upregulation of 1840 transcripts of HSCs (FDR $P < .05$, $|FC| > 2$). MeRIP-seq detected 29833 RNA molecules underwent m6A modification. Through integration of MeRIP-seq and RNA-seq datasets, we identified that in HSCs, 2674 transcripts were downregulated by m6A modification and 1479 transcripts were stabilized by m6A modification (FDR $P < .05$, $|FC| > 1.8$). Gene Set Enrichment Analysis (GSEA) of the transcripts downregulated by m6A revealed that they were enriched in the biological processes, such as Inflammation, carcinogenesis, Epigenetics Transcription, Cellular signaling and fibrosis. Similarly, the transcripts stabilized by m6A were enriched in the biological processes, such as inflammation, epigenetics, carcinogenesis, and metastasis. A targeted proteomics identified that HSC secretion of 22 cytokines/chemokines was altered by knocking down ALKBH5. Among them, 8 were modified by m6A, and 14 were not regulated by m6A. Moreover, in the cytokines/chemokines modified by m6A, 1 were downregulated whereas 7 were upregulated by m6A modification.

Conclusion: M6A critically regulates HSC activation induced by TGFβ. Aberrant m6A modification induced by ALKBH5 targeting suppresses HSC activation, altered HSC transcriptome and HSC secretion of cytokines/chemokines important for shaping the hepatic tumor microenvironment

11: Vav1 promotes pancreatic tumor cell invasion via GLS1-dependent glutamine metabolism FT

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy due in part to metabolic rewiring and a high rate of metastasis. Pancreatic tumors are particularly dependent upon glutamine (Gln) as a nutrient source for multiple metabolic pathways. To adapt to low levels of glutamine, PDAC cells upregulate the Rac1-dependent process of macropinocytosis, which internalizes extracellular macromolecules to scavenge them as a glutamine source. The proto-oncogene Vav1 is an activator of the potent Rac/Cdc42 signaling cascades that regulate actin dynamics. Vav1 expression is significantly increased in PDAC, with high Vav1 correlating with worse overall survival. Here we uncover a novel Vav1-mediated regulation of glutamine metabolism in PDAC cells.

While Vav1 is a potent activator of Rac1, surprisingly, Vav1 expression inhibited macropinocytosis in PDAC cells. Intriguingly, genomic and metabolomic analysis revealed that high Vav1 expression correlates with increased dependence on Gln/Glu (glutamate) metabolism. Indeed, Vav1-dependent macropinocytosis was rescued by cell permeable glutamate, confirming disrupted Gln/Glu balance in Vav1 knockdown cells. RPPA screening indicated a Vav1-dependent effect on GLS1KGA protein (glutaminase1, KGA isoform), which mainly converts Gln to Glu in mitochondria. Mechanistically, we discovered that Vav1 modulates GLS1KGA localization substantially mis-localized away from the mitochondria. Functionally, we demonstrated that knockdown of Vav1 alters 13C Gln-derived metabolic flux into the TCA cycle as well as a deficiency in the level of the antioxidant glutathione (GSH), which utilizes glutamate for its synthesis. Consequently, targeting Vav1 resulted in increased levels of cellular and mitochondrial reactive oxygen species (ROS) and increased sensitivity to lipid peroxidation. Notably, dual targeting of both glucose and glutamine pathways showed significant synergistic effects on cellular viability in Vav1 expressing cells.

Here we established a new tumor-promoting role for Vav1 via Gln/Glu regulation and uncover a novel subcellular regulation of mitochondrial glutamine metabolism. Our findings establish Vav1 orchestrates glutamine metabolism by controlling the GLS1KGA mitochondrial localization. Importantly, Vav1 functions as a signaling adapter in glutamine metabolism, providing a potential therapeutic strategy for PDAC patients with high Vav1 expression.

12: Single cell transcriptional atlas of acute myeloid leukemia samples reveals inflamed state of leukemia stem cells [FT]

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Abstract

Acute myeloid leukemia with TP53 mutations (TP53Mut AML) is a rapidly fatal blood cancer subtype with a high risk of relapse and a survival rate of less than 10% over two years. TP53Mut leukemia stem cells (LSCs) are cancer cells resistant to chemotherapy because they persist in a dormant cell state. LSCs can reawaken and support activation of the LSC self-renewal gene program (LSC-SR), leading to disease relapse. Identification of therapeutic targets to target and block self-renewal would prevent disease progression and relapse in TP53Mut AML.

Prior work has demonstrated that TP53Mut AML has an inflamed microenvironment characterized by dysfunctional T cells. Previously, we found that mutant p53 protein directs the expression of inflammatory mediators including the NFkB/STAT signaling pathway in human AML cell lines and primary human AML samples. Furthermore, both mutant p53 and the NFkB/STAT pathway have been implicated in enhanced LSC function in AML. Therefore, we hypothesize that mutant p53 confers self-renewal in TP53Mut AML via the activation of NFkB-mediated inflammatory pathways. The transcriptional profiles of human AML have been widely published, but have not been reported for TP53Mut AML. Therefore, we performed scRNA-sequencing and constructed a single cell transcriptional atlas of human TP53Mut and wild-type AML samples for the UMN research community.

To understand critical clinical features, we focus on identifying specific cell types that are responsible for these features. We applied our previously developed novel method, Single Cell Correlation Analysis, to pinpoint LSC-SRs. The observation that TP53Mut LSCs are transcriptionally distinct from other AML cells motivates pathway analysis comparing TP53Mut and wild type LSCs. Consistent with our earlier findings, we found that LSCs from TP53Mut AML samples display significant and consistent dysregulation of the NFkB-mediated inflammatory pathways, which may be important for enhanced LSC function driven by mutant p53 proteins.

To validate key elements of the atlas, we used Cytometry by Time-of-Flight to measure quantitative proteins levels in single cells, matched from samples in the transcriptional atlas. We compared the levels of LSC cell surface proteins in each sample to the level of gene expression we detected transcriptionally, demonstrating experimental validation of the atlas. Notably, the inflammatory pathways we highlight can be therapeutically targeted. Our data suggests that targeting these pathways may be an effective strategy to kill TP53Mut AML LSCs.

13: HOXC6 Splicing Produces an Oncogenic Isoform Promoting Head and Neck Squamous Cell Carcinoma [FT]

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Abstract

Human papillomavirus (HPV)-negative head and neck squamous cell carcinoma (HPV- HNSCC) remains a cancer type with limited effective targeted therapies, which are currently restricted to anti-EGFR and anti-PD1 agents. In addition, only two clinically relevant biomarkers are currently available: HPV (or its surrogate p16) and PD-L1, both applicable to a limited subset of patients with HNSCC. Despite extensive genomic sequencing efforts, no new targeted therapy or biomarker has been approved for HNSCC in the past two decades. Our research addresses this critical gap by focusing on alternative splicing events (ASEs), which can profoundly alter gene function independently of mutations and represent an under-explored mechanism of oncogenesis. We hypothesize that ASEs in HPV- HNSCC contribute to tumor progression and can serve as biomarkers and therapeutic targets. To test this, we analyzed RNA-Seq data from The Cancer Genome Atlas (TCGA) and institutional cohorts and identified ASE in Homeobox (HOX) C6 HOXC6. HOXC6, a homeoprotein transcription factor belonging to the HOX family, regulates embryonic development, differentiation, and signaling pathways like TGF-β/SMAD, PI3K/AKT, and WNT. Our previous study indicated an oncogenic role of HOXC6 in HPV- HNSCC; however, the underlying mechanism of HOXC6-driven HPV- HNSCC lies nascent. ASEs of HOXC6 generate distinct isoforms with diverse biological functions. While the HOXC6 transcript variant 1 encodes the primary isoform (HOXC6-1), variant 2 differs in the 5′ UTR coding region and produces a shorter N-terminal isoform (HOXC6-2). To systematically characterize splicing variability, our previous work developed Splice Expression Variability Analysis (SEVA), enabling the detection of differential isoform usage across tumor subtypes. Functional validation revealed that although these isoforms are frequently upregulated in tumors, the spliced isoform (HOXC6-2) is associated with poor clinical outcomes and drives oncogenic phenotypes by promoting proliferation and migration. In conclusion, our findings highlight the impact of ASE in modulating HPV- HNSCC oncogenesis and reveal the oncogenic role of HOXC6-2 in HPV- HNSCC progression. Detection of oncogenic isoforms offers a path toward identification of candidate novel biomarkers, while targeting isoform-specific mechanisms underscores the importance of developing therapeutic strategies. This work introduces a paradigm shift in understanding HPV- HNSCC biology and opens new avenues for biomarker discovery and treatment development.

14: Lysosomal lipid metabolism promotes tumor cell invasion through local energetics and membrane lipid remodeling [FT]

Nooren, R., *Johnson, K.M., Marley, M.G., Rozeveld, C.N., Gibbard, D., Ortiz Correa, Z., Gedik, M.E., Espe, T., Hitosugi, T., Lanza, I., Brownfield, D.G., Liu, J., McNiven, M.A., Razidlo, G.L.*

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Abstract

A promising therapeutic strategy against cancer involves targeting metabolic vulnerabilities to arrest tumor growth. However, few studies focus on the metabolic drivers of tumor cell invasion. Here we report that lipid catabolism by cytosolic versus lysosomal lipases have both overlapping and distinct metabolic and functional consequences contributing to pancreatic cancer cell invasion. Lysosomal lipid droplet catabolism by lysosomal acid lipase (LAL) promotes cancer cell invasion through the formation and stabilization of invadopodia and subsequent degradation of the extracellular matrix. In addition to modulating cellular energetics, lipidomics analysis also revealed a role for lipid droplet catabolism in regulating levels of cholesterol and membrane phospholipids. Further, we demonstrate spatial regulation of lysosomal lipid catabolism at invasive structures, which contributes to both localized ATP levels and membrane cholesterol concentrations at invadopodia. In summary, lipid droplet catabolism by LAL and cytosolic lipases have both shared and distinct consequences for lipid remodeling and cellular energetics during the earliest stages of invasive dissemination.

15: Spatial mapping of brain-tropic gene signatures in cutaneous melanoma [FT]

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Abstract

Metastatic melanoma is an aggressive form of skin cancer characterized by early dissemination of tumor cells. Disseminated cells acquire organotropic molecular programs to create a pre-metastatic niche, enabling survival and colonization at secondary sites such as the brain. The acquisition of these gene signatures and their spatial distribution within primary cutaneous melanoma remain poorly defined. We utilized spatial transcriptomics to compare malignant melanoma (MM) with melanoma in-situ (MIS) to identify gene signatures associated with metastatic potential and brain tropism. Differential expression analysis revealed four key gene signatures (GS) that included cellular junction genes (GS1), proliferation and pro-survival genes (GS2), Ras-related protein genes linked to exosome secretion (GS3), and ribosomal protein large/small subunit genes (GS4). Notably, GS3 and GS4 have been previously associated with circulating melanoma cells and brain metastasis progression. The MM cells had reduced expression of GS1 but high expression of GS2 compared to MIS cells consistent with enhanced invasive and survival capacity. The GS3 and GS4 were upregulated in MM cells, suggesting acquisition of organotropic molecular programs prior to dissemination. Spatial mapping of melanoma cells in MM samples at epidermis/upper dermis, mid-dermis and edge margin sites based on gene activity scores for GS1-4 was done. The gene activity score for GS1 was significantly higher in melanoma cells in the epidermis/upper dermis region while GS2, 3 and 4 scores were high in the mid-dermis region. These findings suggests that a subset of melanoma cells within the mid-dermis region may harbor gene signatures associated with metastatic competence and potential brain tropism. Spatially resolved identification of these high-risk cellular niches may stratify patient population based on their risk for brain metastasis and inform targeted therapeutic strategies.

16: Engineered bone marrow hydrogel platform development to study breast cancer dormancy [SA]

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Abstract

Estrogen receptor-positive breast cancer (ER+) tumor cells can remain in the body for years as disseminated tumor cells (DTCs) after a patient enters remission. DTCs are single cells typically residing in the bone marrow that can evade the immune system and exhibit a dormant phenotype. The only established in vitro dormancy model is the Matrigel-on-Top (MoT) assay. Matrigel creates reproducibility issues since it is animal-derived, has batch inconsistencies, and its mechanical properties cannot be changed. Polyethylene glycol (PEG) hydrogels have been used as a synthetic material for studying dormancy in vitro due to their highly tunable polymer network. Current hydrogel dormancy models encapsulate metastatic cancer cells inside nonporous gels using UV polymerization as a method of inducing dormancy. To better recapitulate the bone marrow niche microenvironment, we are developing an engineered bone marrow hydrogel model with relevant stiffnesses and ECM peptides. 10% w/v PEG-RGD hydrogels were fabricated, and D2.0R cells were seeded on 10% PEG-RGD, Matrigel, and TCPS to determine if MoT assay results could be replicated in PEG-RGD hydrogels. After 7 days on each substrate, PEG-RGD and Matrigel had similar cell counts and morphology compared to TCPS. Our results indicated that D2.0R cells are capable of dormancy on top of PEG-RGD and Matrigel while remaining proliferative on TCPS. Baseline mechanical characterization experiments were performed on varying w/v% PEG hydrogels and Matrigel. Bone marrow has defined Young's moduli (E) throughout its three niches: 0.3 kPa, 5 kPa, and 30 kPa. Gels were measured using shear rheology, and the resulting storage modulus values calculated E. 20% w/v PEG gels were 27.7815 kPa, 10% w/v PEG gels were 18.6915 kPa, 5% w/v PEG gels were 0.609592 kPa, and Matrigel was 1.01920 kPa. Notably, stiffness on 20% w/v and 5% w/v gels recapitulate in vivo bone marrow niche stiffness in the endosteal niche and central marrow, respectively. 5% w/v stiffness is comparable to Matrigel stiffness, but interestingly, D2.0R cells became dormant on a stiffer substrate. This result could foreshadow the potential influence biochemical properties have on DTC dormancy compared to stiffness alone. Engineering the bone marrow niche both biophysically and biochemically using PEG hydrogels will eliminate variability present in the MoT assay, enhance reproducibility, and increase the physiological relevance of in vitro dormancy models.

17: NALCN regulates 'normal' stem cell escape and dissemination from gastric epithelium [FT]

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Abstract

Metastasis remains the leading cause of cancer-related deaths, yet the earliest cellular events that initiate dissemination are poorly understood. Increasing evidence suggests that epithelial cells can disseminate independently of malignant transformation, implicating disrupted tissue homeostasis as a critical early step toward metastatic competence. The sodium leak channel NALCN has emerged as a regulator of epithelial retention: it is downregulated in gastric cancer stem cells, and its genetic deletion promotes dissemination of both malignant and non-malignant epithelial cells. We therefore hypothesised that NALCN maintains gastric stem cell niche retention and epithelial organisation under homeostatic conditions. To test this, we conditionally deleted Nalcn in Prom1^{+} gastric stem cells in mice. Loss of NALCN resulted in fewer gastric glands which presented dysmorphic and disrupted epithelial organisation. Live tissue explant imaging further revealed aberrant retrograde migration of Nalcn-deficient stem cells, which bypassed canonical upward movement along the gland axis and instead migrated basally toward the submucosa. Consistent with niche escape, glands isolated from Nalcn-deficient tissue formed fewer and smaller organoids, suggesting depletion of the resident stem cell pool. To assess conservation in human tissue, we depleted NALCN in human gastric epithelial spheroids, where loss drove invasive protrusion into extracellular matrix. Together, these findings demonstrate that NALCN may maintain epithelial homeostasis by coupling membrane potential to adhesion and niche retention. Its disruption allows gastric stem cells to escape their niche and engage dissemination-permissive migration programmes. Mapping these early dissemination routes may reveal how cancer co-opts normal stem cell behaviour and identify potential targets for intercepting metastasis at its earliest stages.

18: A Novel Total-RNA NGS Library Preparation Platform for Low-Input and Degraded Cancer Specimens

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Abstract

Background: Molecular profiling of cancer specimens is often constrained by poor nucleic acid quality and limited input. Biopsies, FFPE tissues, liquid biopsies, exosomes/extracellular vesicles, plasma cell-free RNA/DNA, and other clinically valuable materials are frequently fragmented, degraded, or available only in picogram quantities. Current methods described as "total RNA" sequencing often fail to capture the full RNA spectrum, require separate workflows for small and long RNAs, and introduce transcript-length, abundance, and degradation-dependent bias.

Methods: We developed a patented NGS library-preparation platform that functions as a true total-RNA sequencing technology by capturing all RNA species ≥20 nt), enabling simultaneous sequencing of small and long RNAs from the same low-input sample. The method was evaluated using picogram quantities and degraded RNA templates and compared with established commercial or published workflows at similar sequencing depth.

Results: Our platform consistently produced 2-10X higher usable data yield, broader transcriptome coverage, and high reproducibility from low-input and highly degraded RNA. In proof-of-concept studies, the method detected >200,000 unique transcripts without apparent saturation and captured 20,000–100,000 more annotated transcripts than established methods. The platform showed negligible bias for transcript length or abundance, supporting more accurate and quantitative measurement of transcript copy numbers. Libraries generated from as little as 500 pg total RNA retained broad recovery of both short and long RNA species with reduced technical variation across input amounts and sample quality.

Conclusions: This technology addresses a major bottleneck in cancer genomics by enabling robust transcriptome profiling from precious, degraded, or previously unusable clinical specimens. Its ability to recover broad, quantitative RNA information from biopsies, liquid biopsies, FFPE tissues, exosomes, and cell-free RNA/DNA can improve biomarker discovery, tumor subtyping, treatment-response monitoring, and translational oncology research. The platform's low-input performance, automation compatibility, and broad transcriptome recovery also support future applications in single-cell and spatial transcriptomics.

19: Identifying a novel population of epithelial cells in the blood and bone marrow of normal mice and humans

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Abstract

Epithelial cells have been identified in the blood and bone marrow (BM) of cancer patients using epithelial cell adhesion molecule (EpCAM) as a marker. These EpCAM+ cells are generally referred to as Circulating Tumor Cells (CTCs) and used diagnostically to determine cancer prognosis. However, some EpCAM+ cells can also be found in the blood and BM of the controls from normal healthy human donors. The presence of these EpCAM+ cells in the blood and BM of healthy individuals has yet to be studied in a consistent way. We investigated these EpCAM+ cells in the blood and BM of healthy mice and humans to determine why they express EpCAM, a known epithelial marker and if they have characteristics of stem cells.

Epithelial markers, such as EpCAM and pan-cytokeratin, were used on blood and BM of healthy mice and humans using flow cytometry, immunofluorescence microscopy, and a Krt1-14;mTmG transgenic mouse model. Epithelial cells in healthy individuals were first identified and isolated via flow cytometry using epithelial cell adhesion molecule (EpCAM). These EpCAM+ cells were confirmed to express keratin using immunofluorescence microscopy in Krt1-14;mTmG transgenic mice. Human blood samples had 0.18% ± 0.0004 of EpCAM+ cells (SEM; n=7 biological replicates, 4 experimental replicates). In human BM, 3.53% ± 0.006 (SEM; n=3 biological replicates, 4 experimental replicates) of mononuclear cells were EpCAM+. In mouse blood, EpCAM+ cells constituted 0.45% ± 0.0006 (SEM; n=2 biological replicates, 4 experimental replicates), and in mouse BM, 5.17% ± 0.001 (SEM; n=3 biological replicates, 4 experimental replicates) were EpCAM+. In mice, the EpCAM+ cells were immunoreactive to pan-cytokeratin as determined by IF microscopy. Results were confirmed using Krt1-14;mTmG transgenic mice, with low (8.6 native GFP+ cells per 106 cells analyzed; 0.085% of viable cells), but significant numbers ($p<0.0005$) of GFP+ cells present in normal murine BM that were not the result of randomness compared with multiple negative controls. Further, EpCAM+ cells were found to have varying levels of CD44 and CD45 expression, known migration and leukocyte markers.

Using flow cytometry and immunofluorescence microscopy, we have found the presence of cells positive for EpCAM, CD44, CD45, and some keratin expression within normal human and mouse blood and bone marrow. From our flow cytometry data, these EpCAM+ cells seem to be a heterogeneous population with varying levels of CD45 expression.

We decided to pursue single cell RNA sequencing as a method for further elucidating the function and phenotype of these EpCAM+ cells. In single cell data from normal mice and human BM, we have found the presence of cells with transcripts for EpCAM, CD44, CD45, and some keratin expression. Using multiple publicly available datasets, including the Tabula Muris and Tabula Sapiens, along with a dataset from 25 healthy human donors, which comprised about 80,000 single cells, we found that the EpCAM+ cells comprised about 3.5% of the human BM cells from healthy donors. Using these data and our own experimental data, we have consistently found a heterogeneous population of EpCAM+ cells in the blood and BM of normal healthy mice and human individuals.

Virtual: A Multi-Omics Immune Signature for Predicting DCIS Progression to Invasive Breast Cancer: An AI-Driven Discovery Approach [SAA]

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Abstract

Background: Less than one-third of untreated cases of ductal carcinoma in situ (DCIS), which makes up 20–25% of screen-detected breast malignancies, develop into invasive breast cancer (IBC) within ten years. Most patients undergo intensive treatment regardless of their actual risk because practitioners are unable to accurately predict which lesions will progress. The biological complexity of DCIS heterogeneity is not fully captured by current risk measures, such as nuclear grade, ER/HER2 status, and commercial gene panels, which function on a single molecular layer. We hypothesised that a molecularly invasive subgroup concealed within histologically homogeneous DCIS could be uncovered by multi-omics AI integration, providing useful information that single-layer classifiers are unable to.

Methods: Gene expression, DNA methylation, pathway activity, and immune cell composition are the four molecular data types that we combined from five publically accessible patient datasets (GEO; n = 415 total, including 46 DCIS, 102 IBC, and normal breast tissue samples). A 41-gene core invasive signature was confirmed in two separate validation cohorts from the 2,996 substantially changed genes found by differential gene expression analysis across 102 IBC/DCIS samples. After being trained to differentiate IBC from DCIS, a hybrid multi-modal transformer, an AI architecture that simultaneously learns associations across all data layers, was used to discover a molecularly distinct high-risk subgroup within the DCIS samples. Multivariate logistic regression was used to independently validate epigenetic risk in a different clinical outcomes cohort (GSE281307; n = 185 DCIS patients with 10-year follow-up).

Results: The transformer outperformed traditional machine learning techniques in every metric, achieving an AUC of 0.98 for IBC vs. DCIS classification (sensitivity 95%, specificity 76%). The 41-gene core signature revealed a consistent biological narrative: eight epithelial identity genes, such as basal keratins and hemidesmosomal collagen COL17A1, were lost in IBC, while 33 stromal activation genes, such as fibrillar collagens, matrix metalloproteinases, and cancer-associated fibroblast markers, were elevated. When the trained model was applied to DCIS samples, it was shown that, despite having the same histological classification, 32.6% (15 of 46) had an invasive-like molecular profile, placing them at the IBC boundary in dimensionality reduction analysis. Beyond age and nuclear grade, progression to invasive illness was independently predicted in the independent clinical cohort by membership in a high-risk DNA methylation cluster (odds ratio 2.40, 95% CI 1.27-4.67, p = 0.008). Additionally, the HER2-enriched subtype (OR 3.71, p = 0.028) was an independent predictor. The top molecular drivers were determined by AI-based feature attribution (SHAP analysis) to be TAC1, COL4A3, PIGR, SFRP1, and MAMDC2, all of which have known functions in invasion biology.

Conclusions: According to multi-modal AI integration, about one-third of DCIS cases have an invasive disease molecular fingerprint that is undetectable by conventional pathology. Patients at a 2.4-fold increased risk of progression are independently identified by epigenetic cluster membership, which may be measured from regular biopsy samples using conventional methylation arrays. As far as we are aware, this is the first multimodal transformer framework to independently validate and identify an invasive-like subgroup within DCIS. These results lay the groundwork for molecularly guided risk classification, which may help guide decisions regarding tighter monitoring in high-risk patients and therapy de-escalation in low-risk DCIS. The first stages toward therapeutic translation are integration with spatial transcriptomics data and prospective validation in a clinical trial cohort.

20: Single Cell Resolution of an Epigenetic Signature of Persister Tumor Cells in Ovarian Cancer [FT]

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Abstract

Cancer can recur when a subset of tumor cells, or persister cells, are able to survive therapy and eventually re-enter the cell cycle. We hypothesized that an epigenetic signature underlies a drug-tolerant persister state, characterized by transcriptional and chromatin accessibility changes that promote survival of residual cancer following chemotherapy. To identify persister cells, we performed single-cell multiomic profiling (snRNA+snATAC from the same nuclei) on a cohort of non-malignant fallopian tube, treatment-naive, and neoadjuvant chemotherapy (NACT)-treated high-grade serous ovarian cancer (HGSOC) samples. Using a cohort of 14 patients we were able to define transcriptional states associated specifically with malignancy and characterize the major changes between non-malignant and tumor cells. We also identified differences in gene expression and open chromatin between naïve and NACT patient tumors following chemotherapy. The epigenomic analysis revealed activity of several DNA-binding factors that are both enriched upon chemotherapy and high in resistant tumors prior to treatment. From this analysis, we identified an epigenetic signature that precedes expression and defines the persister state. Targeting persister cells in vitro revealed that the top ranked chromatin regulators enriched in our persister signature (CREBBP/EP300 and BRD2/4) can be targeted to synergize with chemotherapy. This epigenetic signature also correlated with chemotherapy sensitivity and resistance using patient-derived xenograft models of HGSOC and publicly available data from scATAC in metastatic HGSOC disease. Finally, our analysis also revealed that persister cells are primed for chemotherapy tolerance via chromatin accessibility highlighting an epigenetic predisposition for chemo-resistance. In summary, we identified a signature of drug-tolerant persister cells in high grade serous ovarian cancer using chromatin accessibility and transcriptome single-cell profiling.

21: Stability Selection and Bidirectional Validation for Transportable Prognostic Biomarker Discovery in Oral Squamous Cell Carcinoma [FT]

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Abstract

Background: Prognostic gene signatures for oral squamous cell carcinoma (OSCC) inferred from omics sequence data frequently fail to replicate across independent cohorts, partly due to unstable feature selection in high-dimensional omics data. Stability selection provides a theoretically grounded framework for identifying genes that are selected reproducibly, but its implementation requires careful consideration of the underlying base selection procedure.

Methods: We developed a multi-stage pipeline combining randomized lasso stability selection with penalized Cox regression and neural network validation. RNA-seq expression data from two independent OSCC cohorts including TCGA (n=339, 153 events) and ORIEN (n=1,112, 450 events) were preprocessed through gene harmonization, batch correction, and unsupervised variability filtering (60,486 to 4,448 genes). Stability selection was performed independently within each cohort using 50 random subsamples with per-gene randomized penalty weights. Per-cohort stable genes were combined into a union set, which was validated through bidirectional cross-cohort evaluation and external validation on two independent Affymetrix microarray datasets (GSE41613, n=97; GSE42743, n=71).

Results: Stability selection identified 12 genes stable in TCGA and 14 in ORIEN, with only one gene (DDIT4) overlapped in both cohorts, yielding a 25-gene union set. Bidirectional cross-cohort validation achieved mean C-indices of 0.620 (Cox) and 0.626 (neural networks). External validation confirmed gene set transportability with median C-indices of 0.625 (GSE41613, HPV-negative) and 0.671 (GSE42743). Kaplan-Meier analysis demonstrated significant risk stratification across all four cohorts (all log-rank $p < 0.01$).

Conclusion: Randomized lasso stability selection identified a transportable 25-gene prognostic signature for OSCC. Although only DDIT4 (hypoxia–mTOR axis) was stable in both cohorts, the per-cohort gene sets converged on shared biological themes including metabolism, cell migration, inflammation, and DNA damage response supporting biological coherence beyond gene-level overlap.

22: TMEM198 and Cancer-Related Signaling

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Abstract

Background: TMEM198 (Transmembrane Protein 198) is a poorly characterized membrane protein located on chromosome 2q35, and is made up of 360 aa. It is known to promote LRP6 phosphorylation via casein kinase recruitment, activating the canonical Wnt/β-catenin signaling pathway, a well-established driver of colorectal (rectosigmoid cancer) and other cancers. Despite this, TMEM198 remains understudied relative to other Wnt pathway components.

Methods: Comparative genomic analysis using multiple sequence alignment (T-Coffee) across 13 vertebrate species, protein topology prediction (TMHMM), promoter analysis (JASPAR), and expression profiling using NCBI GEO, Human Protein Atlas, and cBioPortal somatic mutation data.

Results: TMEM198 is highly conserved across mammals, birds, fish, and amphibians (T-Coffee consistency score: 96), with strong conservation in transmembrane domains and variability in the disordered regions (Dis1/Dis2). Topology prediction confirms 6–7 transmembrane helices. Promoter analysis identified binding sites for ZNF436 and Runx1, transcription factors dysregulated in multiple cancers. Expression profiling shows broad tissue expression in (fetal) brain and endocrine tissues. Somatic mutation analysis reveals TMEM198 alterations across stomach, colorectal, endometrial, and bladder cancers.

Conclusion: TMEM198 possesses structural features relative to its role and Wnt-driven cancer progression. When overexpressed, cancer, especially Rectosigmoid cancer, making it more likely. This data suggest further investigation as a potential oncogenic co-regulator in metastatic contexts.

23: Dissection of the role of cholesterol biosynthesis genes in hepatocellular carcinoma using CRISPRi and single-cell RNA sequencing [SAA, FT]

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Abstract

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related mortality worldwide, with incidence tripling over the past four decades. Concurrently, metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as a dominant driver, accounting for 50% of current HCC cases. Reprogramming of cholesterol metabolism is a hallmark of MASLD-driven HCC progression; however, the precise functional contributions of individual enzymes within the de novo cholesterol synthesis pathway remain poorly defined.

To systematically characterize these regulatory nodes, we utilized an engineered Huh7 CRISPR interference (CRISPRi) model to silence each of the 30 cholesterol synthesis genes, followed by single-cell RNA sequencing (scRNA-seq). Our screen revealed that knockdown (KD) of individual pathway components yielded highly divergent, unique transcriptional signatures rather than a uniform metabolic shutdown. From these distinct profiles, we selected two candidate genes for functional validation: MVK and DHCR7.

Mevalonate kinase (MVK), a key upstream enzyme, has been implicated in suppressing ferroptosis during HCC progression. We demonstrate that MVK KD significantly reduces cell size during S-phase and induces robust cell cycle accumulation in the G1 phase. Notably, this proliferation arrest occurs independently of canonical mTOR signaling, unveiling an alternative, uncharacterized regulatory axis.

Conversely, targeting 7-dehydrocholesterol reductase (DHCR7), which catalyzes the final step of cholesterol biosynthesis, induced a starkly different phenotype. DHCR7 KD promoted expression of genes associated with hepatic differentiation. Subsequent functional analyses indicate that this phenotypic shift is likely driven by the accumulation of the substrate 7-dehydrocholesterol; its metabolites, Vitamin D and 25-hydroxy-7-dehydrocholesterol, subsequently activate Vitamin D Receptor (VDR) and Liver X Receptor (LXR)-mediated differentiation pathways.

Collectively, our single-cell functional genomics approach demonstrates that inhibiting specific nodes within cholesterol biosynthesis does not merely deplete cholesterol, but triggers discrete, exploitable biological phenotypes, ranging from cell cycle arrest to differentiation. These distinct molecular mechanisms present novel, tailored therapeutic strategies to combat HCC progression.

24: Improving High-Grade Cervical Lesion Detection Using Quantum-Inspired Deep Learning Optimization

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Abstract

Background/Objectives: Automated cervical cytology classification remains challenging due to complex multicellular morphology and subtle visual differences between diagnostic categories such as low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL). Inaccurate classification may lead to delayed diagnosis and treatment. This study introduces an Enhanced Schrödinger Algorithm (EnSRA), a quantum-inspired optimization framework designed to improve deep learning performance and reliability for cervical cytology screening.

Methods: EnSRA integrates Adaptive Quantum Superposition and Golden Ratio Perturbation strategies to optimize seven hyperparameters of a ResNet50 model trained on the Brown Multicellular ThinPrep cervical cytology dataset, consisting of 601 images across three classes (NILM, LSIL, and HSIL). Stratified data splitting (70/15/15) and image augmentation techniques were applied during training. Model performance was evaluated using accuracy, precision, recall, and F1-score, and compared against state-of-the-art convolutional neural network and transformer architectures.

Results: The proposed EnSRA-optimized ResNet50 achieved a test accuracy of 98.89% with a macro F1-score of 0.9889, outperforming advanced architectures including ViT-B/16 (90.00%), Swin Transformer-Tiny (95.56%), and DenseNet-121 (97.78%). The model demonstrated high computational efficiency with an inference time of 0.57 ms per image. Importantly, the framework achieved 100% recall for HSIL cases, resulting in zero false negatives in the test cohort, highlighting its potential clinical value for high-grade lesion detection and screening safety.

Conclusions/Implications: The proposed EnSRA framework significantly improves cervical cytology classification through adaptive quantum-inspired hyperparameter optimization. By enhancing diagnostic accuracy while maintaining high sensitivity for clinically significant lesions, this approach demonstrates strong potential for AI-assisted cervical cancer screening and clinical decision-support applications. These findings further emphasize the importance of intelligent optimization strategies in advancing reliable and scalable AI systems for cancer diagnostics.

25: Implications of polymorphic tRNA arginine in fragmentation and predicted structures as a potential driver of tumor onset and metastasis in murine breast cancer models [FT]

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Abstract

Transfer RNA fragments (tRF) are a recently described non-coding RNA type able to alter cancer progression and metastasis. While the mitochondrial genome encodes only 13 proteins, 22 mitochondrial tRNA are expressed and display polymorphisms (SNP) in tRNA-arginine's (mt-tR) D-loop across mouse strains. To assess the role of mitochondrial genomes in mouse models of cancer, we developed Mitochondrial Nuclear eXchange (MNX) mice (PMID: 23924350) combining nuclear and mitochondrial genomes from C3H/HeN, C57/Bl6J, BALB/cJ, and FVB/N mice. Wildtype and MNX mice across strains display differential tumor latency and metastatic potential, depending upon the mtDNA in tumor and/or stromal cells. Tissue metabolomic profiles were predominantly dependent on the nuclear genome but surprisingly did not vary significantly in MNX mice. SNP in mt-tR correlated with phenotypic changes, compelling the hypothesis that tRF from mt-tR were responsible for differential tumor latency and metastases. Polymorphic tRF were discovered in liver and lungs of normal mice and in mammary tumors derived of MMTV-PyMT mice in strain- and tissue-dependent abundance patterns. Full-length mt-tR expression was relatively consistent in all mouse strains, but expression of the predominant tRF (52-54nt) varied by strain. tRF from FVB mitochondria were most abundant compared to other strains when assessed across the C57/Bl6J nuclear background and correlated with the shortest latency of tumor development. Full-length mt-tR was modeled using AlphaFold3 to identify structural differences. Predicted interactions between the D- and T-loops of folded full-length mt-tR varied due to D-loop polymorphisms. We hypothesize that D-loop SNP may regulate mt-tR stability and/or T-loop cleavage to generate tRF. Further, we hypothesize that differential D-T loop interactions drive tRF generation which leads to variation in mitochondria-driven differences in tumorigenesis and metastasis. Ongoing work tests these hypotheses by identifying tRF interactors. Support: Metavivor, Natl Fndn Cancer Res., NIH.

26: Shrink Polymer–Based Ultra-Sensitive Biosensor for Detection of the Pancreatic Cancer Biomarker CA19-9

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Abstract

Early detection of pancreatic cancer remains a major clinical challenge, partly because sensitive biomarker testing often relies on centralized laboratory assays that require complex instrumentation and trained personnel. Carbohydrate antigen 19-9 (CA19-9) is a clinically established biomarker associated with pancreatic cancer and other gastrointestinal malignancies, but rapid, low-cost, and sensitive detection platforms are still needed to support earlier diagnosis and disease monitoring. Here, we report a shrink polymer-based electrochemical biosensor for ultrasensitive detection of CA19-9. The sensor was fabricated by depositing Cr/Au electrodes onto a shrink polymer substrate followed by thermal shrinking to generate highly wrinkled micro/nanostructured gold surfaces. This structural engineering increases the effective electrode surface area and improves interfacial electron transfer, enabling amplified electrochemical signal transduction without relying on complex nanomaterial synthesis. The biosensing interface was constructed through DTSP-mediated surface functionalization, streptavidin immobilization, and biotinylated CA19-9 aptamer attachment. Electrochemical impedance spectroscopy and cyclic voltammetry confirmed successful stepwise functionalization, while differential pulse voltammetry was used for quantitative CA19-9 detection.

The shrink polymer-based electrode achieved a low detection limit of 0.1 U/mL and showed a broad linear response from 0.1 to 10,000 U/mL with strong correlation (R^2= 0.99). Compared with planar gold electrodes, the microstructured shrink polymer electrode exhibited approximately threefold higher sensitivity, demonstrating the benefit of surface structuring for biomarker sensing. Validation using pancreatic cancer cell culture supernatants showed strong agreement with ELISA measurements, with over 93% accuracy. These results suggest that shrink polymer-structured electrodes provide a simple, scalable, and cost-effective platform for sensitive CA19-9 detection. With further validation in clinical samples, this strategy may support future point-of-care diagnostic technologies for pancreatic cancer screening and monitoring.

27: Metabolic Reprogramming Enhances CAR T-Cell Control of Established Tumors and Blocks Metastatic Dissemination [SA]

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Abstract

Despite major advances in CAR T-cell therapy for hematologic malignancies, durable eradication of established solid tumors and prevention of metastatic dissemination remain critical barriers to effective therapy. We have found that pharmacologic activation of CAR T cells with the cellular energy sensor AMP-activated protein kinase (AMPK) enhances CAR T-cell metabolic fitness and improves clearance of leukemia cells in vitro and in vivo, significantly prolonging survival in tumor-bearing mice. We hypothesized that similar benefits might be seen with the treatment of solid tumors.

The ability of malignant cells to persist within localized tumor sites, and subsequently disseminate to distant organs, underscores the need for models that provide both solid tumor architecture and the potential for systemic spread. To evaluate the impact of AMPK activation on CAR T-cell control of established tumors, mice were implanted subcutaneously with Nalm6 cells mixed 1:1 with Matrigel to generate localized lymphoma-like tumors. Twenty-one days later, mice received CD28-based anti-CD19 CAR T cells which had received either treatment with the AMPK agonist, Compound 991, or vehicle control. Tumor burden was monitored longitudinally using bioluminescence imaging, and survival was assessed through day 96 post-CAR T cell injection. Treatment with 991-conditioned CAR T cells enhanced clearance of established tumors, improved disease control, and prolonged survival ($P < 0.01$; n=10-11 mice/group) in tumor-bearing mice compared with conventional CAR T-cells.

To directly assess the ability of CAR T cells to impact metastatic dissemination, cells were recovered from the original tumor bed and the spleen, either at the time of euthanization (for vehicle-treated control animals) or at day 96 for long-surviving recipients of 991-treated CAR T cells. Receipt of 991-treated CAR T cells increased the intratumoral accumulation of both CD4+ and CD8+ CAR T cells at the time of analysis. Furthermore, while CD19+ZsGreen+ Nalm6 cells were present in the original tumor bed in 2/10 991-treated CAR T cell recipients, there were no Nalm6 tumors cells recovered from the spleen in the two tumor positive animals, indicating effective prevention of systemic dissemination. In contrast, all five tumor positive animals treated with vehicle control CAR T cells exhibited both residual tumor burden and evidence of metastatic spread to the spleen. These new findings highlight a critical role for metabolic reprogramming in preventing metastatic dissemination of established disease in dynamic solid tumor models.

28: Ongoing Preclinical Study of an AAV vaccine in Spontaneous Canine Model of Oral Melanoma: Safety and Efficacy Evaluation

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Abstract

Lung metastasis is the main cause of death of companion dogs diagnosed with oral melanoma (OM), an aggressive cancer that begins in the mouth's mucosal tissue. OM is treated through surgical removal of tumor followed by intense radiotherapy mirroring human melanoma onset and treatments. This makes OM diagnosed dogs a great preclinical model to test our multivalent optimized AAV6 anti-melanoma vaccine. This vaccine is composed by a mutated AAV6-S663V to prolong the vectors lifespan by avoiding proteolytic degradation in target cells, ensuring delivery of optimized expression cassette and eliciting the corresponding immune response to avoid cancer reappearance. We used 1e12vg or 3e12vg of each antigen to create a triple-valent vaccine of low or high dose respectively, that delivers 3 known melanoma antigens, glycoprotein 100 (Gp100), tyrosinase (Tyr) and tyrosinase related protein 1 (TRP-1). Previous work has proven the vaccines effectiveness in melanoma mouse models, effectively promoting protection against lung metastasis. The ongoing preclinical trial for this multivalent AAV vaccine against oral melanoma is reaching 3.5 years. To date, 10 dogs with I-III stage OM have been administered the vaccine, 6 males and 4 females ranging between 6.5 and 14.3 years of age. 6 animals received a 3x10 12 vg/ dog dose while 4 animals received 9x1012 vg/ dog dose. These animals are followed for up to 9 months to evaluate blood, liver and renal function parameters to determine safety and efficacy of vaccine. Prior to vaccine administration, preexisting neutralizing antibodies (NAb) against AAV6 capsid were determined to meet eligibility criteria of 1:2-1:8 titers for enrolment. After vaccine administration, NAb titers increased 1 to 4 weeks in most dogs, and at later time points started to decline, opening the possibility of vaccine boosting. With blood PBMCs, specific immune reaction against vaccine antigens (gp100, TYR and TRP-1) were analyzed through IFN-γ ELISPOT assay determining CD8+ T-cell activation at different time points, revealing three types of response, early, late and the non-responders (only one dog). Animals were also monitored through blood biochemistry, and chest radiography to track metastasis in the lungs. Globulins and white blood cell (WBC) count were tracked for a sign of inflammatory responses. Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (ALS) were evaluated for liver function, as were creatinine and blood-urea-nitrogen (BUN) evaluated for renal function. The values of these parameters suggested no evidence of liver or renal problems related to the vaccine administration.

29: Characterization of NALCN Expression in Mammary Epithelial Cells

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Abstract

NALCN is an ion channel known to regulate cell migration, proliferation, and apoptosis. Recent studies suggest that NALCN also functions as a tumor suppressor gene and may serve as a prognostic biomarker or therapeutic target. This study aimed to examine NALCN expression in malignant and nonmalignant epithelial cell line models to better understand its potential role in tumorigenesis and metastasis. Using immunofluorescence staining and confocal microscopy, NALCN expression was evaluated in mouse breast cancer cell lines (4T1, EO771, and MMTV-PyMT) and in a nonmalignant control (C57BL/6). The results revealed that NALCN is expressed in both malignant and nonmalignant epithelial cells, with notably higher expression in the triple-negative breast cancer (TNBC) cell lines 4T1 and EO771. These findings suggest that epithelial–mesenchymal transition (EMT) may contribute to increased NALCN expression and metastatic potential in TNBC models. Future studies will involve quantifying NALCN expression using Western blotting, immunocytochemistry, qPCR, and immunohistochemistry to further characterize its role in breast cancer progression and therapeutic targeting.

30: RNA-sequencing derived Radiosensitivity Index (RSI) is not a predictor of local recurrence after postoperative radiotherapy in resected brain metastases FT

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Abstract

Introduction: Postoperative radiotherapy is an adjuvant therapy for brain metastases(BrMs) that improves local recurrence(LR). The radiosensitivity index(RSI) is a 10-gene index of pan-cancer molecular radiosensitivity previously validated for predicting radiotherapy response in clinical datasets. In this external validation study, we evaluate RSI as a predictor of local recurrence(LR) after radiotherapy in resected BrM.

Methods: Patients from University Health Network Toronto(UHN) and McGill University who had a resected BrM and subsequently underwent whole brain radiation(WBRT) or stereotactic radiosurgery(SRS). RSI was calculated using a ranked linear regression model from the weighted expression of 10 radiosensitivity genes, with higher values corresponding to radioresistance. Radiophenotype was determined using a previously validated RSI cutoff of <0.375 to identify radiosensitive tumors. LR was defined as recurrence in the resection cavity and overall survival(OS) was measured from time of BrM diagnosis.

Results: 106 BrM patients were included, comprising 29 breast, 37 GI, 33 lung, and 7 melanoma primary sources. Mean age at surgery was 59.2 years, 31.1%(n=33) were male, 67.9%(n=72) had WBRT, 23.6%(n=25) had a subtotal resection, and 30.2%(n=32) had postoperative chemotherapy. Overall, 34.0%(n=36) patients had LR. Patients with breast BrMs had median RSI 0.70(IQR:0.55-0.75, 10.3% radiosensitive) with 48.3% LR(n=14); gastrointestinal BrMs had median RSI 0.43(IQR: 0.35-0.46, 29.7% radiosensitive) and 24.3% LR(n=9); lung BrMs had median RSI of 0.67(IQR:0.66-0.71, 9% radiosensitive) and 33.3% LR(n=11); melanoma BrMs had median RSI of 0.72(IQR:0.53-0.92, 0% radiosensitive) and 28.6% LR(n=2). After adjusting for demographics, primary organ, radiotherapy, resection extent, among others, RSI did not predict LR(p=0.56) or OS(p=0.61).

Discussion: RSI did not predict either LR or OS in patients with surgically resected BrMs status post radiotherapy, further establishing the need for improved predictive scores in this population.

31: Mechanistic Investigation of Ruthenium-Avobenzone mediated Cytotoxicity[FT]

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Abstract

Ruthenium β-Diketonate complexes have emerged as promising chemotherapeutics due to their observed bioactivity against tumors. We assessed the cytotoxicity of synthesized Ruthenium (p-cymene)(avobenzone)(PTA) and Ruthenium (p-cymene)(avobenzone)(Cl) complexes on liver cancer and kidney embryonic cells, as well as against the bacterial species Escherichia coli and Staphylococcus aureus. Using cytotoxicity assays, we screened candidate Ruthenium complexes and determined the IC50 value for effective candidates. Based on these data, computational methods were used to demonstrate potential mechanisms for toxicity. Computational modeling at the r2SCAN-3c level of theory indicates a low likelihood of avobenzone detachment in aqueous solutions. In contrast, a far greater likelihood exists for chloride detachment versus PTA detachment in aqueous media. Computational docking of the complexes was performed in two segments of DNA from a human colon cancer (Caco-2) cell line: a guanine-rich portion and a randomly-selected portion. The most stable poses of [Ru(p-cymene)(avobenzone)]Cl in each DNA segment had favorable binding energies with the avobenzone positioned at least partially within the major groove. These data indicate that the mechanisms of toxicity involve intact Ru-Avobenzone complexes.

32: The H3.3 Accessome Gap: S31P Depletion Drives Structural Tail Sequestration, Mitotic Lockout, and Chromosomal Instability in Pediatric Glioma [SAA, FT]

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Abstract

Background: Histone regulation of tumor initiation and progression is fundamentally relevant to all cancer types. Pediatric high-grade gliomas are universally lethal malignancies driven by histone mutations H3.3 K27M and G34R/V. Traditional paradigms attribute midline oncogenesis to enzymatic PRC2 inhibition by K27M, causing global H3K27me3 loss. However, we recently challenged this methyl-centric dogma. We demonstrated that H3.3 K27M reduces the affinity for the kinase CHK1, suppressing phosphorylation of the adjacent, H3.3 exclusive Serine 31 (S31P). Crucially, our in vivo mouse models show that an H3.3 S31A phospho-null mutant phenocopies K27M to drive high-grade gliomas and catastrophic chromosomal instability (CIN) while maintaining normal global H3K27me3 levels. This indicates that physical tail architecture can act as a primary driver of oncogenesis.

Methods: To investigate the structural consequences of S31P loss, we performed quantitative CUT&RUN profiling, mass spectrometry, and Western blotting on isogenic heterozygous CRISPR mutant cell lines modifying 1 of 4 H3.3 alleles (H3.3 K27M and H3.3 S31A) against wild-type controls.

Results: Our data unmasked a stark biochemical paradox. While mass spectrometry and Western blot confirm global levels of H3K27me3 and H3K4me3 remain stable in S31A lines (unlike the global H3K27me3 reduction in K27M), CUT&RUN profiles for these same marks showed a dramatic, quantitative reduction in both mutant lines. This manifests as a systemic collapse of peak amplitude rather than a localized loss of specific loci. We term this an "Accessibility Gap," signaling a topological lockout where the H3.3 N-terminus becomes sterically shielded. Our prior work establishes that K27M prevents CHK1 P of S31P, which directly correlates with a failure to maintain inter-kinetochore tension, leading to increased lagging chromosomes and the propagation of CIN during mitosis. Our current data demonstrates that this loss of the S31P phospho-wedge induces a genome-wide accessibility gap that physically blocks diverse epigenetic interactors, including the readers of H3K27me3. Whether this architectural occlusion reflects a stable, conformationally trapped ""tucked"" state or a transient reduction in overall tail availability remains under active investigation.

Conclusion: S31P serves as an essential conformational gatekeeper; its loss triggers an architectural collapse of the H3.3 N-terminal tail, driving rapid tumor evolution. Crucially, by sterically locking out H3K27me3 readers and inducing a mitotic lockout, the S31A model isolates a purely structural axis that phenocopies the downstream signaling failures of K27M, proving tail sequestration alone drives gliomagenesis. Preserving the S31P phospho-wedge offers a structural stabilization strategy to keep the H3.3 accessome open, suppress CIN, and extend the post-biopsy survival window for secondary interventions like CAR-T cells.

33: Primary cilia promote resistance to EGFR tyrosine kinase inhibitor, osimertinib, in non–small cell lung cancer [FT]

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Abstract

Patients with advanced Non–small cell lung cancer (NSCLC) and mutations in epidermal growth factor receptor (EGFR) benefit from EGFR tyrosine kinase inhibitors (TKIs). Osimertinib, a third-generation EGFR TKI, is standard first-line therapy for EGFR-mutated NSCLC, but most patients develop resistance to it. Here, we demonstrate that increased formation of primary cilia, microtubule-based sensory organelles, is associated with osimertinib-refractory NSCLC progression. EGFR-mutated, osimertinib-resistant human NSCLC cells had increased cilia formation and acetylation of α-tubulin and reduced histone deacetylase 6 (HDAC6) activity compared to their osimertinib-sensitive counterparts. HDAC6 inhibition increases cilia formation in osimertinib-sensitive NSCLC cells, and overexpression of exogenous HDAC6 sensitized osimertinib-resistant NSCLC cells to osimertinib's anti-proliferative effects. Because intraflagellar transport (IFT) proteins are essential for primary cilium formation and function, we knocked down IFT88 in osimertinib-resistant NSCLC cells, which reversed osimertinib resistance in orthotopic and subcutaneous mouse models of lung cancer. Mechanistically, increased sodium influx during osimertinib-induced inhibition of EGFR signalling promotes cilia formation through sustained HDAC6 inactivity and greater α-tubulin acetylation. Inhibition of sodium influx with dibutyryl-cAMP decreased cilium formation, increased sensitivity to osimertinib, and reduced tumor progression in mice bearing osimertinib-resistant lung tumors. Collectively, our findings suggest that enhanced primary cilium formation mediates EGFR TKI resistance and that targeted inhibition of ciliogenesis may prevent or overcome osimertinib resistance.