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1. Organotypic Brain Slice Cultures: A Versatile Platform for Tumor Engraftment and Analysis

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Brain cancers are notoriously challenging due to their heterogeneity, drug resistance, and invasive nature, leading to poor prognoses. To combat them,

effective drug development necessitates models that closely mimic human tumor responses. Unfortunately, traditional in vitro methods often fall short in replicating the complexity of these cancers. To overcome these limitations, we have developed an organotypic brain slice culture (OBSC) model that mirrors in vivo tumor characteristics within a functional tumor microenvironment (TME), providing rapid and relevant results. Our OBSC model allows for the measurement of tumor growth, morphology, and invasion rates. Tumors engrafted onto OBSCs can be subjected to therapeutic treatments and analyzed for drug efficacy. In a 4-day assay, we successfully engrafted a variety of brain cancer cell lines, including low-passage lines that are typically difficult to establish in vivo. These cell lines maintained their specific invasion patterns, interacted with the OBSC microenvironment, and exhibited drug response patterns consistent with in vivo behavior. Notably, invasive and metastatic cell lines migrated outward on OBSCs, while densely growing lines contracted inward. To validate the OBSC model as a model for the TME, we treated MB231BR, LN229, and U373 tumor lines with TR107, an experimental ClpP inhibitor derived from ONC201. This therapeutic family requires a functional TME to achieve maximum efficacy, which is absent in standard in vitro conditions. While in vitro experiments showed incomplete tumor kill, treatment on OBSCs resulted in complete tumor elimination, highlighting the OBSC model's ability to replicate TME-based tumor responses. In conclusion, OBSCs offer a valuable platform for studying brain cancers, accurately replicating tumor behavior and therapeutic responses within the TME. This model holds significant potential for advancing drug development and enhancing our understanding of brain cancer biology, ultimately improving patient outcomes.

2. Crystallization-Induced Diastereomer Transformations of Donor-Acceptor Cyclopropanes

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Chiral nonracemic donor-acceptor (D-A) cyclopropanes are important synthetic building blocks for stereospecific and stereoselective transformations. Herein, we report the first crystallizationinduced diastereomer transformation (CIDT) of D-A cyclopropanes. Achiral Lewis acids catalyze rapid epimerization of aniline-substituted D-A cyclopropanes through reversible C-C bond cleavage. Formation of the conjugate acid anilinium salt concurrently slows epimerization and enhances the crystallinity of the cyclopropanes. In contrast with the well-precedented Lewis acid activation paradigm, we also describe a CIDT wherein solvent-promoted epimerization of a diastereomeric mixture of aniline-substituted cyclopropanes proceeds spontaneously in the absence of a Lewis acid. The crystallinity of the free

aniline is sufficient to promote the CIDT without the need for an acid/base switch. Products can be isolated by filtration without the need for further purification. Furthermore, the products of these reactions can be easily derivatized using the aniline as a functional handle to access electronically diverse, diastereopure cyclopropanes. By establishing stereochemistry using a C2-symmetric malonate, a small library of diastereo- and enantiopure D-A cyclopropanes was synthesized. We envision that this unique approach will offer a practical and scalable method to access such compounds for medicinal or process chemistry applications.

3. Assessing the Performance and Stability of Nitrocyclocondensation Reactions on Silicon Electrodes for CO2 Reduction

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The attachment of molecular catalysts onto semiconductor surfaces is crucial for enhancing the performance and durability of hybrid photoelectrodes. Covalent modification of native silicon faces many challenges, particularly from the formation of insulating SiO2 layers at unreacted sites, which inhibit photocatalysis by creating trap states. While there have been many different techniques use to immobilize molecules to silicon, silicon-carbon bond-forming chemistries have been the most explored. However, these molecules are still susceptible to oxidation and molecular backfilling is necessary to passivate residual unreacted silicon atoms. Silicon-nitrogen bonds (355 kJ/mol) are thermodynamically stronger than silicon-carbon bonds (318 kJ/mol) but are relatively unexplored compared to carbon as an attachment technique for silicon. The Tepalakov group et al. have demonstrated monolayer modification of silicon via cyclocondensation of nitro- or nitroso-functional groups under ultrahigh vacuum with no nitrogen desorption up to 1000K. The thermodynamic stability of this oxynitride attachment indicates a promising strategy for improving silicon interfaces in CO2 reduction photoelectrodes.

This study optimized a solution-phase nitro-cyclocondensation reaction, which is performable under benchtop conditions, and evaluated the stability of silicon oxynitride films during CO2 reduction. Initial experiments with 4-nitrophenyl ferrocene (4-NpFc) quantified surface loading and electrochemical reversibility, comparing surfaces with and without nitromethane (NM) backfilling using cyclic voltammetry. X-ray photoelectron spectroscopy (XPS) characterized silicon-

nitrogen bond distributions and oxidation states after functionalization.

A photoelectrode was fabricated by coupling a carboxylic acid Re(bpy)(CO)3Cl catalyst derivative to an amine-terminated silicon film via standard amide chemistry, followed by NM backfilling. Bulk electrolysis at -1.75 V (Ag/AgCl) under one sun illumination demonstrated the conversion of CO2 to CO, quantified by gas chromatography, showcasing the catalytic efficiency of the system and the effect of backfilling with NM.

4. Structure Activity of β-Amidomethyl Vinyl Sulfones as Covalent Inhibitors of Chikungunya nsP2 Cysteine Protease with Anti-alphavirus Activity

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Despite their widespread impact on human health there are no approved drugs for combating alphavirus infections. The heterocyclic β-aminomethyl vinyl sulfone RA-0002034 (1a) is a potent irreversible covalent inhibitor of the alphavirus nsP2 cysteine protease with broad spectrum antiviral activity. Analogs of 1a that varied each of three regions of the molecule were synthesized to establish structure-activity relationships for inhibition of Chikungunya (CHIKV) nsP2 protease and viral replication. The covalent warhead was highly sensitive to modifications of the sulfone or vinyl substituents. However, numerous alterations to the core 5-membered heterocycle and its aryl substituent were well tolerated and several analogs were identified that enhanced CHIKV nsP2 binding. For example, 5-(2,5-dimethoxyphenyl)pyrazole (1o) and 4-cyanopyrazole (8d) analogs exhibited kinact/Ki ratios >9,000 M-1s-1. 3-Arylisoxazole 10 was identified an isosteric replacement for the 5-membered heterocycle, which circumvented the

intramolecular cyclization that complicated the synthesis of pyrazole-based inhibitors like 1a. The accumulated structure-activity data was used to build a ligand-based model of the enzyme active site, which can be used to guide the design of covalent nsP2 protease inhibitors as potential therapeutics against alphaviruses. Isoxazole 10 demonstrated inhibition of nsP2pro, remarkable proteome-wide selectivity, and has the chemical stability required of a high-quality chemical probe. We have characterized 10 as a covalent chemical probe for Chikungunya nsP2 protease with antialphaviral activity and proteomewide selectivity.

5. Inhibition of Parp7 enhances anti-tumor systemic immune responses when combined with radioimmunotherapy in breast cancer

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More than half of all cancer patients receive radiotherapy (RT) during their cancer experience. DNA damage induced by RT can initiate a pro-inflammatory immune response within the tumor microenvironment (TME), yet this response is not uniformly observed across all tumors. Four *Trp53-/- Balb/c* breast cancer syngeneic allograft models with low tumor mutational burden and resistance to dual ICI (anti-PD1 and anti-CTLA4) were implanted with bilateral tumors and randomly divided into four treatment cohorts: untreated, RT, ICI, or RT+ICI, where RT (8Gyx3) was administered to one tumor. An abscopal response is an immune mediated anti-tumor response at a location distant from the radiation field. While they are relatively rare in patients, they do occur, particularly in the context of RT+ICI. Two lines exhibited abscopal responses to RT+ICI (i.e., “abscopal models”), whereas the other two did not (i.e., “non-abscopal models”). The purpose of this study is to investigate mechanisms of enhancing the frequency and duration of an abscopal response. To identify gene expression patterns associated with abscopal responding breast tumors, we performed spatial transcriptomic analysis of tumors 10 days after initiating treatment using the GeoMx whole transcriptome assay targeting panCK+ tumor cells and tumor adjacent CD45+ immune cells (N>5 for each treatment group and tumor type). Unsupervised hierarchical clustering was performed to identify gene expression patterns across areas of interest. Genes selectively induced by RT+ICI in abscopal models that were not induced in non-abscopal models were identified using two-tailed t-test and FDR correction for

multiple testing (FDR<5%). DeSEQ2 and GSEA analysis were performed using publicly available mouse gene sets (Broad Institute). Hierarchical clustering of immune related genes in CD45+ segments of RT+ICI treated tumors exhibiting an abscopal response revealed a global shift in gene expression profiles after treatment. GSEA analysis of CD45+ segments from abscopal models shows that RT+ICI treatment results in enrichment of the interferon mediated signaling pathway including interferon alpha ($p<0.0001$), interferon beta ($p<0.0001$), type II interferon response ($p<0.0001$), and antigen processing and presentation ($p<0.0001$). Bulk RNA-sequencing of tumors cultured in vitro and treated with RT shows increased expression of the PARP family of proteins in abscopal models. Inhibition of Parp7 induces 1000-fold increase in IFN-b expression in abscopal models. Abscopal models treated with RT+ICI+Parp7i exhibit an increased abscopal response ($p<0.001$) and extended survival ($p<0.001$) compared to RT+ICI alone. These results indicate that inhibition of Parp7 may improve clinical outcomes in ICI resistant breast tumors when combined with RT.

6. An Adenylosuccinate Lyase in Antibiotic Biosynthesis and Biocatalysis

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The aspartase/fumarase superfamily of enzymes catalyze C-N or C-O bond cleavage from succinate containing compounds, releasing fumarate as the common product.[1] Adenylosuccinate lyases are members of this superfamily that typically catalyze C-N bond cleavage of adenylosuccinate leading to formation of adenosine monophosphate (AMP) in purine biosynthesis.[2] Our lab identified FlcB, an adenylosuccinate lyase in the biosynthesis of a copper-containing antibiotic, fluopsin C, from the bacterium *Pseudomonas aeruginosa*. In the first step of fluopsin C biosynthesis, FlcB catalyzes the formation of a C-S bond between cysteine and fumarate, generating an (*R*)-stereocenter.[3] The enantioselectivity of FlcB differs from other adenylosuccinate lyases and therefore makes it an intriguing subject for future study. We showed that FlcB is permissive toward various thiol- and amine-containing substrates. Kinetic parameters of FlcB were measured and essential catalytic residues were identified. Together, these findings shed light on the mechanism of FlcB. The enantioselective C-S bond formation catalyzed by FlcB has potential applications in biocatalysis.

7. Poly(2-oxazoline) Polymers to Improve Lipid Nanoparticle-Based Drug Delivery

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Lipid nanoparticles (LNPs) have emerged as an eNective and versatile means of delivering genetic material to target cells within the body for applications like vaccines and cancer therapeutics. PEGylated lipids help prolong LNP circulation time by decreasing particle aggregation and reducing kidney-mediated clearance from the blood. However, PEG polymers are prevalent in other shelf products and can induce an immune response in people with prevalent anti-PEG antibodies, and have also been observed to cause accelerated blood clearance upon subsequent dosing. In this study, we explore the substitution of PEG with poly(2-oxazoline) polymers, which are both bioinert and highly modifiable, to improve LNP-based delivery of genetic materials. Through cationic ring opening polymerization, we synthesize poly(2-ethyl-2-oxazoline) and poly(2-methyl-2- oxazoline) polymers of diNerent chain lengths and then conjugate them with phospholipids for incorporation within LNPs. We show that the physicochemical properties of poly(2-oxazoline) LNPs, such as size, charge, stability, and encapsulation efficiency, are comparable to traditional PEG LNPs. We then formulate poly(2-oxazoline) LNPs encapsulating firefly luciferase mRNA and compare their transfection efficacy *in vitro* and *in vivo* with PEGylated LNPs. Finally, we establish polymer-dependent differences in the hard protein corona of LNPs in human plasma, implying serum-dependent properties which could influence LNP fate and targetability. Overall, we find that poly(2-oxazoline) LNPs to be functional vehicles for gene delivery while opening the door for potentially beneficial chemical modifications to current LNP structures.

8. Decoding the Dynamic Architecture of Exonuclease I in DNA Mismatch Repair: A New Perspective from Atomic Force Microscopy

Exonuclease I (EXOI) is integral to the DNA mismatch repair (MMR) pathway, a process crucial for the maintenance of genomic stability. Despite structural insights provided by crystallography of EXOI's catalytic N-terminal domain, the highly disordered C-terminal domain and the dynamic behavior of the protein remain poorly understood. Notably, more than half of EXOI is predicted to be disordered, raising questions about the conformational flexibility that governs its function, particularly in the MMR pathway. We are utilizing atomic force microscopy (AFM),

both in-air and in-solution, to probe the conformational dynamics of EXOI. In parallel, circular dichroism (CD) experiments are being employed to elucidate the secondary structure of EXOI, offering complementary insights into its structural transitions. Additionally, in the presence of MutS, a key MMR protein, and with or without nucleotide, we are exploring how EXOI modulates DNA structure and the broader implications of its interaction with MMR components. By examining these dynamic interactions and conformational states, our study aims to elucidate the mechanisms by which EXOI contributes to mismatch repair. These findings will provide critical insights into the structural flexibility of EXOI, which may ultimately inform our understanding of its regulatory roles in MMR. Through a combination of AFM and CD, we are building a comprehensive picture of the structural transitions that govern EXOI's function in this essential pathway.

9. Stereoconvergent Transformations of β-Dicarbonyl Compounds

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Transformations of chiral, racemic starting material is a heavily investigated area of research, including manifolds such as kinetic resolution, dynamic kinetic resolution, and stereoablative methods. One manifold that is relatively underdeveloped is using a stereodivergent transformation followed by a stereoconvergent transformation to access enantio- and diastereopure products. We propose a merged asymmetric catalysis-crystallization induced diastereomer transformation (AC-CIDT) sequence, which follows this manifold. A stereodivergent conjugate addition of a boronic acid is performed on a racemic α-monosubstituted γ,δ-unsaturated β-keto amide, using Rh(I)-catalysis and a chiral diene ligand. The resulting addition product is then subjected to base-mediated CIDT conditions, isolating the product through filtration and avoiding the use of time- and resource-intensive purification methods. Various aryl boronic acids and β-keto amides were screened, leading to isolation of single diastereomers of product.

10. Targeting B7H3-Driven Tumor Growth with CAR-T Cell Therapy in Mouse Models of Obesity and Triple Negative Breast Cancer

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Background:

Triple negative breast cancer (TNBC) is one of the most difficult to treat breast cancer subtypes and is promoted by obesity, in part by obesity-driven immunosuppression. Hence, novel treatments are needed. B7H3 is an immunosuppressive transmembrane protein often overexpressed in cancers, including TNBC, but its role in obesity-driven cancer growth is unknown. B7H3 is a promising target for chimeric antigen receptor (CAR)-T cell therapy given its restricted expression in adult tissues. Such B7H3-targeting CAR-T cell therapies are enticing as they control solid tumor growth where the tumor microenvironment (TME) strongly dictates CAR-T cell efficacy. Critically, no mechanistic studies testing the impact of obesity on CAR-T therapy exist. Here, we sought to determine how obesity alters the antitumor effects of direct B7H3 suppression and impacts B7H3 CAR-T cells.

Methods:

Female C57BL6/NCrl mice consumed either 60% kcal fat diet or a 10% kcal fat diet for ≥15 weeks to generate diet-induced obese (DIO) or control mice, respectively. Once DIO mice reached body weight of 45g, mice underwent orthotopic injection of E0771 cells either i) overexpressing B7H3 (E0771_B7H3HI) or ii) B7H3 targeting shRNA or nontargeting control. Mice with E0771_B7H3HI tumors received lymphodepleting chemotherapy followed by infusion of B7H3 CAR-T or non-transduced (NT) T cells. Immune cells were isolated from tumor and spleen and T cells were measured using flow cytometry. Tumor growth was monitored using calipers.

E0771_B7H3HI cells were cultured with B7H3-targetting CAR-T cells for 3 days to determine in vitro killing efficacy. Interleukin 2 and interferon gamma were measured to assess CAR-T cell activation. After coculture, cells were stained with viability dye and CD3 and measured using flow cytometry.

Results:

B7H3 enables TNBC growth in murine models of obesity and is upregulated by obesity-associated cytokines. B7H3-expressing TNBC is targetable with CAR-T cell

therapy. Obese, but not lean mice, had significant reduction in tumor volume when administered B7H3 knockdown E0771s relative to controls, and flow cytometry results indicate more T cells in knockdown tumors relative to controls (figure 1). Flow cytometry and ELISA results confirm CAR-T cell efficacy in vitro (figure 2). In vivo B7H3-targetting CAR-T therapy equally controls tumor growth in lean and obese models of TNBC (figure 3).

Conclusion:

B7H3 is a potential promoter of tumor growth in obese models of TNBC, likely via an immunoregulatory mechanism. Results also indicate B7H3 CAR-T cell therapy controls tumor growth in lean and obese mouse models of TNBC, revealing that CAR-T therapy may overcome the obese TME to control solid tumor growth.

11. Characterizing Paraneoplastic Pemphigus proteins that are highly expressed in most Triple Negative Breast Cancers.

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Breast Cancer (BC) is a leading cause of cancer-related deaths among women in the United States, estimating around 42,250 deaths in 2024. BCs are heterogeneous and can be categorized into 3 clinical phenotypes based upon the expression of three therapeutic targets that are ER, PR, and HER2; these 3 clinical subtypes are ER+/HER2-, HER2+, and Triple-Negative Breast Cancers (TNBC) that lack all three. BC can be further classified according to the gene expression patterns, which identify 5 molecular subtypes. Around 80% of TNBCs belong to the Basal-Like Breast Cancer (BLBC) molecular subtype and compared to other subtypes, BLBCs have the worst prognosis and survival. During our investigations of the biology of BLBCs, we performed a supervised analysis comparing BLBC versus all other molecular subtypes using mRNA and protein expression data of 109 human BC tumors and we identified 7 functionally related genes that have both mRNA and protein levels significantly higher in BLBCs. These 7 genes/proteins show two intriguing characteristics that are 1) the majority are components of inter-cellular adhesion junction Desmosome, and 2) all are known autoantibody targets in a rare autoimmune disease called Paraneoplastic Pemphigus (PNP) which is almost always associated with an underlying cancer. The central hypothesis of our study is that these 7 genes highly expressed in BLBC (hereafter referred to as PNP genes/proteins) are contributing to

BLBC 1) tumor progression and/or 2) tumor immune responses.

Multiple human BC cohorts (e.g. TCGA-BRCA) with clinical and transcriptomic data are utilized to study the role of PNP genes in BC. We are using CRISPR/Cas9 gene knockout and lentiviral-based gene overexpression in human BC cell lines followed by *in vitro* and *in vivo* characterization to evaluate if PNP genes are involved in BLBC tumor progression. To investigate the potential role of PNP genes in BLBC immune responses, we have designed peptide microarrays containing overlapping 15mer peptides spanning each PNP protein, and other known autoantibody targets, and we are investigating if human BC patient sera contain autoantibodies (IgG) against these PNP proteins. We have assayed 21 BC patients and 1 normal healthy sera so far and when analyzing the antibody reactivity on the peptide level, even though there is a limited overlap across patients, 110 PNP peptides were targeted by serum IgG autoantibodies of at least 4 BC patients assayed. Out of which, 9 PNP peptides were targeted uniquely by serum autoantibodies of 4 or more TNBC/BLBC patients. These 9 peptides were not targeted by the Luminal/HER2-E subtype BC patient nor by normal individual sera. We are assaying more TNBC/BLBC patient sera along with sera from other BC subtypes and normal healthy individuals to validate our findings and comparing the presence of PNP autoantibodies to important clinical and molecular features including patient outcomes (e.g. survival) and the presence or absence of tumor immune cell infiltrates. Collectively, this study will dissect the role that these 7 PNP genes may play in BLBC tumor biology and inform whether these genes and their associated molecular pathways could be used as potential biomarkers for early detection or predicting clinical outcomes and in discovering better treatment strategies for BLBC.

12. Translating Stereoconvergent Catalysis of Small Molecules to Polymer Science

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The relative (tacticity) and absolute (asymmetry) stereochemistry of polymers has profound effects on their thermomechanical, optical and assembly properties. Recently, there is a growing interest to expand the diversity of stereoregular materials with monomers containing pre-installed chiral centers as racemic mixtures. However, state-of-the-art kinetic resolution methods limit yields to 50% for an asymmetric, isotactic polymer following the assumption that the stereochemistry of the monomer translates directly to the polymer. This contrasts with stereoconvegent catalysis in organic chemistry, which has revolutionized synthesis by interrupting the transfer of chiral information from substrate to product, providing a clear platform for catalysts to access enantiopure compounds

from racemic mixtures in up to 100% yield.

In this lecture, I will discuss the development of a stereconvergent chain-growth polymerization that converts 100% of a racemic monomer to an isotactic, asymmetric polymer. To accomplish this, we leveraged the reactivity of transition-metal pi-allyl complexes derived from vinyl aziridines using rhodium catalysis, which ablates and resets chiral centers during polymerization. I will describe the effect of ligand structure on stereoselectivity, which enabled access to polymers with up to 17 kDa molar mass while maintaining high meso selectivity. Mechanistic studies support stereoconvergence occurs at a faster rate than polymerization, which is attributed to isomerization of the prochiral rhodium pi-allyl intermediate prior to propagation. Post-polymerization modifications of the stereoregular polymers identified tacticity and polymer asymmetry-induced property changes, including the identification of a new stereocomplex.

13. Optimizing Opioid Receptor RNA Quantification Informs Transcriptome-Wide Improvements in RNA Sequencing Experiments

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Opioids produce pain relief and adverse side effects by binding the mu-opioid receptor (MOR; encoded by *Oprm1*) in different types of neurons. Targeting specific opioid-sensitive cell types may lead to powerful analgesics without adverse side effects. *Oprm1* splice variants and non-neuronal expression may also contribute to adverse opioid side effects, but conflicting evidence questions their biological significance. Single-cell RNA-sequencing (scRNA-seq) can characterize *Oprm1*+ cellular heterogeneity, inform RNA structure, and reveal cell type-specific treatment targets in opioid-sensitive cells. While generating a brain-wide atlas of *Oprm1*-expressing cells, we discovered a ~20-fold difference in *Oprm1*+ cell proportions based on whether intronic reads were included during RNA alignment. We determined that intronic *Oprm1* reads primarily originate from an incomplete 3'UTR annotation in the primary MOR-1 isoform and from long-range *Oprm1* splice variants. Bioinformatic and histological analyses revealed that intronic reads aligned to the 3'UTR likely originate from *Oprm1* RNAs. These reads are accurately quantified if intronic reads are included during sequence alignment and affect both bulk and single-cell RNA-seq. Conversely, we determined that intronic reads from long-range *Oprm1* splice variants are likely caused by a technical artifact. In this case, intronic read inclusion generates false positives, which account for most

Oprm1 reads in cortical neurons and virtually all *Oprm1* reads in non-neuronal cells. We generated a refined gene annotation to retain false negative *Oprm1* RNAs and remove false positive *Oprm1* RNAs. Our annotation refinement increased RNA quantification concordance between sequencing and histological approaches. These *Oprm1* quantification artifacts translate to other species, transcriptomic platforms, and genes, which are correctable using our annotation optimization method.

14. Machine Learning of Three-Dimensional Protein Structures to Predict the Functional Impacts of Genome Variation

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Research in the human genome sciences generates a substantial amount of genetic data for hundreds of thousands of individuals, which concomitantly increases the number of variants of unknown significance (VUS). There are few, if any, computational methods for variants comparable to biological activity predictions. To address this gap, we developed a machine learning method that uses protein three-dimensional structures from AlphaFold to predict how a variant will influence changes to a gene's downstream biological pathways. We have trained state-of-the-art machine learning classifiers that attain accuracies higher than 80%, which have allowed us to identify a set of key protein regions that lead to significant perturbations in c-Myc or NRF2 transcriptional pathway activities.

15. Combined vertical autophagy inhibition and RAS MAPK pathway inhibition as a therapeutic strategy for pancreatic ductal adenocarcinoma.

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PDAC growth is characterized by dependencies on both mutant KRAS signaling and autophagy. Our group and others previously demonstrated that inhibition of the RAF/MEK/ERK pathway in PDAC induces an increased dependence on autophagy.

Combined inhibition of the ERK MAPK pathway and autophagy inhibited the growth of multiple preclinical models of PDAC. Based on these findings, combined ERK/MEK inhibition and hydroxychloroquine (HCQ) is currently under clinical evaluation (NCT03825289, NCT04386057). However, resistance to this combination has already been described. To identify sensitizers to CQ treatment, our lab performed a CRISPR-Cas9 mediated loss of function screen. Surprisingly, we identified multiple autophagy related genes, indicating that concurrent inhibition of two nodes of the autophagy pathway may be a more effective method of inhibiting autophagy in PDAC. We identified, *PIK3C3*, the gene that encodes for VPS34, a protein essential for the nucleation of autophagosomes, as a potential sensitizer to CQ treatment. Inhibition of VPS34 resulted in both decreased autophagic flux and impaired proliferation. Additionally, VPS34 inhibition sensitized cells to CQ treatment, leading to further reduced proliferation. We also found that inhibition of ULK1, a serine/threonine kinase critical for autophagy initiation, further reduced CQ-mediated anti-proliferative effects. We next determined that both VPS34 inhibition and ULK1 inhibition can decrease RAS inhibitor induced autophagic flux. Finally, vertical inhibition of the autophagic pathway via VPS34 inhibition and CQ sensitized PDAC cells to RAS inhibition to further reduce PDAC proliferation. Ongoing studies are aimed at elucidating the mechanism leading to decreased proliferation of combined anti-autophagy and RAS inhibitor therapies.

16. Mre11 Mediates cGAS Activation and Tumor Suppression through ZBP1-Dependent Necroptosis Pathway in Breast Cancer

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Oncogene-induced replication stress leads to endogenous DNA damage, activating the cGAS/STING signaling pathway, a critical player in tumor suppression. The exact mechanism underlying cGAS activation, however, remains unclear due to the continual inhibition of cGAS by its high-affinity interaction with the histone acidic patch (AP), which sterically prevents its activation by double-stranded DNA (dsDNA). In this investigation, we elucidate the significant role of the DNA double-strand break sensor, Mre11, in regulating cGAS activation and thereby inhibiting mammary tumorigenesis. Our data reveal that the binding of the Mre11-Rad50-Nbn (MRN) complex to nucleosome fragments is crucial to liberate cGAS from AP-mediated sequestration, facilitating its subsequent activation by dsDNA. Consequently, Mre11 emerges as a vital component in the cGAS activation process, responding to oncogenic stress, cytosolic dsDNA, and ionizing radiation. Moreover, we highlight the ramifications of Mre11-dependent cGAS activation which fosters ZBP1/RIPK3/MLKL-mediated necroptosis, a vital process in curtailing oncogenic proliferation and breast tumorigenesis. Significantly, our study identifies a strong correlation between the downregulation of ZBP1 in human triple-negative breast cancer and increased genomic instability, suppressed immune response, and adverse patient prognosis. Our findings firmly establish Mre11 as a pivotal mediator connecting DNA damage to cGAS activation, thereby facilitating tumor suppression through ZBP1-dependent necroptosis, offering a promising avenue for targeted breast cancer therapies.

17. Activation of Dithiolopyrrolone Antibiotics by Cellular Reductants

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Dithiolopyrrolone (DTP) natural products are broad-spectrum antimicrobial and anticancer prodrugs that contain a unique bicyclic ene-disulfide that, once reduced in the cell, chelates metal ions and disrupts metal homeostasis. In this work we investigate the intracellular activation of the DTPs and bacterial resistance to the DTP scaffold. We find that the DTP is a privileged scaffold that is activated by several redox proteins and small molecules, reducing the likelihood of high-level bacterial resistance. This work advances the understanding of DTP activation and informs the development of bioreductive disulfide prodrugs.

18. Assessing Toxicological Effects of PFAS Exposures on *In Vitro* Liver Models

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Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds that accumulate in the bloodstream and tissue due to the liver's inability to metabolize carbon-fluorine (C-F) bonds. Prolonged exposure to low concentrations of PFAS mixtures (sub-ppb levels) is thought to increase the risk of hepatotoxicity and disrupt the regulation of metabolic pathways. Recent studies highlighted potential links between cytochrome P450 (CYPs) enzyme activity and PFAS exposures. These findings showcase the unintended consequences of these "forever chemicals" used in many industrial coatings, possibly targeting the nuclear receptors that regulate CYP expression and inhibiting enzyme activity. There is a need for research that elucidates the mechanism by which PFAS alter CYP activity. In this work, *in vitro* liver models were used to develop and validate a PFAS screening pipeline. This pipeline assesses the effects of PFAS exposures on cell viability (calcein-AM), CYP1A enzymatic activity (EROD assay), and potential AHR-mediated mechanism (TCDD inducer and SR1 inhibitor studies). ELISAs for CYP1A1 enzymes were used to determine if differences in expression levels caused altered enzyme activity. The screen focused on six PFAS regulated by the US Environmental Protection Agency (EPA), tested individually (10 µM exposure) or in binary mixtures (10 µM each). The six molecules have varying lengths (C4 – C8) and terminal functional groups (e.g., carboxylic acids vs. sulfonates): PFBS, PFHxS, PFOS, GenX, PFOA, and PFNA. Cell viability was unaffected by six PFAS molecules individually as well as in the fifteen binary mixtures. Sulfonate-containing PFAS showed statistical differences in basal CYP1A activity. Three binary mixtures containing a sulfonated PFAS – PFBS & PFOS, PFBS & GenX, and PFOS & PFNA – also exhibited statistically different CYP1A activity. Preliminary ELISAs indicate that altered CYP1A1 expression levels did not cause these activity differences. Further research will elucidate correlations between the PFAS studied and (i) structure-specificity and/or (ii) receptor-mediated pathways.

19. Universal Detection of SARS-CoV-2 using Cell Surface Sugar

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Inspired by the role of glycans in the virus-host cell entry process, we report the development of GlycoGrip: a glycan-based lateral flow assay for detecting SARS-CoV-2 and its variants. The spike protein on SARS-CoV-2 has high potential as a target antigen for developing highly selective diagnostics. However, its constant and rapid mutation makes it challenging to utilize the spike protein as a target antigen for diagnostics. To overcome this, GlycoGrip leverages glycans to universally capture the SARS-CoV-2 virus. Using glycans in diagnostics offers advantages over conventional immunoassays because glycans can bind to various viral surface proteins, regardless of mutations, through multivalent interactions. This unique feature makes GlycoGrip highly adaptable to emerging variants and other virus types. By integrating computational modeling, we were able to efficiently design and optimize GlycoGrip for selective, sensitive, and robust detection of SARS-CoV-2 and its variants. As new variants continue to arise, we envision GlycoGrip as a promising sensing technology that can be generalized and quickly tailored to address emerging infectious viruses.

20. Nitric Oxide-releasing Liposomes for Treatment of Pulmonary Nontuberculous Mycobacteria

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Non-tuberculosis mycobacteria (NTM) are ubiquitous, opportunistic pathogens that cause severe respiratory infection primarily in elderly and immunocompromised populations. The most common pathogenic strains of NTM include members of the *Mycobacterium avium* complex (MAC) and the *Mycobacterium abscessus* groups. Due to their hydrophobic cellular envelopes, NTM survive within immune cells following phagocytosis, creating a barrier for systemically administered antibiotics, which limits

efficacy. Liposomes are a class of phospholipid nanovesicles that are well-known for drug delivery benefits. Recently, liposomal antibiotic formulations have been developed to treat MAC pulmonary infections. However, their utility against *M. abscessus* pulmonary infections is limited due to fast growth and antibiotic resistance. Nitric oxide (NO) is an endogenously produced reactive free radical that exerts antimicrobial effects against pathogens via several mechanisms of action, and as such is unlikely to elicit resistance. Methyl tris diazeniumdiolate (MD3) is a small molecule that is capable of sustained release of NO, making it an attractive candidate for antimicrobial therapeutics; however, it is a triply negatively charged molecule, making cellular uptake unlikely. Thus, liposomal NO delivery systems may further enhance the utility of NO release for treating intracellular NTM infections. Herein, we describe the development of a novel pulmonary therapeutic that combines the bactericidal properties of NO with the targeted delivery of liposomes. Liposomal formulations were evaluated as a function of pH and lipid composition and optimized for MD3 loading, allowing for bactericidal levels of NO to be released from the system. Two clinically relevant morphologies of *M. abscessus* were selected for in-vitro planktonic and intracellular infection experiments to demonstrate antibacterial efficacy using the NO-releasing liposomes. Furthermore, the cellular uptake of the liposomes was evaluated qualitatively and quantitatively. Preliminary results with altering lipid composition suggest a viable formulation for long-term storage under lyophilization. Future work will focus on functionalizing targeting moieties such as mannose, in order to facilitate liposomal delivery to pulmonary macrophages.

21. Single Molecule Förster Resonance Energy Transfer (smFRET) Reveals Multiple DNA Conformations in GEN-5'flap Complexes.

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Eukaryotic GEN is structure-specific endonucleases that resolves Holliday junctions (HJs) by forming a dimer to induce its endonuclease activity on HJs during homologous recombination. GEN also recognizes and cleaves other substrates such as 5' flaps, double flaps, and replication forks. Most studies, however, only focus on GEN's cleavage on HJ despite higher activity of GEN on 5'flaps than HJs. Our previous biochemical studies suggest that GEN can dimerize on 5'flaps and increase the cleavage rate relative to the monomer. Consequently, in this work, we use single molecule FRET to examine the conformational changes associated with monomer and dimer binding to elucidate the structural mechanism that governs the faster cleavage by the dimer. We find that a binding of a monomer to the flap induces DNA bending, similar to studies with the 5'flap endonuclease FEN1. Notably, however, binding of a second monomer further increases DNA bending. This increased DNA bending is consistent with our previous biochemical studies, which suggest that the rate-limiting

step to cleavage is a DNA conformational change that optimizes the flap position for cleavage. Finally, to examine role of the flap in promoting dimer formation, we characterized the stoichiometry and lifetimes of GEN binding to a systematic series of varying length 5' flaps. Taken together, these data provide a picture of the coordinated series of conformational changes that lead to optimal flap cleavage.

22. N–Si Heterolysis by Chiral (BOX)Cu(OTf)2 Catalysts for the Synthesis of Indole and Carbazole Glycosides

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Chiral Cu(II) bisoxazolines have been shown to catalyze the coupling of acetyl-protected carbohydrates with N-silylated indoles to give the corresponding N-glycosides. Preliminary mechanistic experiments indicated that catalysis occurs through formation of a Cu–indolide complex with concomitant formation of TMS-OTf which together activate the sugar and deliver the indole nucleophile.

23. Visualizing DNA Repair MutL Homologs Using Atomic Force Microscopy to Observe Protein Dynamics and Conformational Changes

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DNA mismatch repair (MMR) is a highly conserved pathway in all organisms. MMR is initiated by MutS and MutL homologues. In humans, MutLα and MutLγ are protein heterodimers that contain ATPase, DNA binding, and endonuclease activity. MutL homologs dimerize via their C-terminal domains, which contain endonuclease activity. The N-terminal domains, which contain DNA binding and ATPase activities, are connected to the C-terminal dimerization domains via a long, disordered linker arm. Biochemical and in-air atomic force microscopy (AFM) studies indicate that MutLα adopts multiple ATP-induced conformational changes, ranging from highly compact to cases where both disordered linker arms are extended. Comparison of the in-air AFM data on MutLα and MutLγ shows that MLH3 (164 kDa) in MutLγ contains an additional

structured domain relative to PMS2 (96 kDa) in MutLα. The MLH3 subunit can form this additional structured domain in the center of its “disordered” linker arm without its binding partner MLH1. To better understand the structure-function relationship and dynamics of these intrinsically disordered MutL proteins, we employed in-air and high-speed in-solution (HS-IS) AFM to characterize the distributions of their conformations and to capture real-time images of these molecules. We use HS-IS-AFM to elucidate the dynamic mechanisms of interconversion between conformational states. Taken together, these data help us to better understand protein dynamics and how their conformational changes may be linked to the functional role of these MutL proteins within their biological pathway.