TOPIC 1: Analytical, Structural, and Glycotechnology Methods

(PT-001) Profiling of O-fucose proteome of POFUT1 and POFUT2 using 6-alkynyl fucose

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Domain-specific O-fucosylation by Protein O-fucosyltransferase 1 and 2 (POFUT1/2) is an unusual yet biologically important glycosylation. Many POFUT1/2 substrates, including Notch receptors, regulate essential biological events. Disrupted O-fucosylation is associated with many human congenital disorders and cancers, underscoring the need to characterize the O-fucose proteome. However, glycoproteomic profiling of intact O-fucosylated peptides remains challenging. O-fucosylated proteins constitute a very small fraction in cells, and unlike other glycan modifications that generate oxonium ions as signatures of glycopeptide for mass spectrometric analysis, O-fucose is frequently observed as a neutral loss, thus complicating the confident assigning of glycopeptides. To address these challenges, we designed an unbiased glycoproteomic workflow for in-depth profiling of POFUT1/2 substrates called "Direct detection of Fucose Analog-Tagged peptides (DidFAT)". We previously demonstrated that 6-alkynyl fucose (6-AF) is efficiently incorporated into POFUT1/2 substrates. Here we leveraged bioorthogonal click chemistry to couple 6-AF with a cleavable biotin linker, enabling NeutrAvidin enrichment and selective chemical release of labeled peptides while leaving a residue Fuc6nyl* tag. The Fuc6nyl* tag displayed unique mass spectrometric features by generating high-intensity, O-fucose-specific diagnostic ions that greatly improved the confident assigning of glycopeptides. In a proof-of-principle experiment using HEK293F secretome with expressed ADAMTS9, the DidFAT workflow robustly enriched O-fucosylated peptides from all predicted sites in ADAMTS9, with over 90% of MS2 spectra containing Fuc6nyl* diagnostic ions. Besides ADAMTS9, a handful of endogenous proteins were unambiguously identified with fucose on their predicted sites, including NOTCH1, ADAMTS3 and NELL1. Moreover, unexpected O-fucose sites were identified on several proteins with noncanonical sequence motifs or domain structures. O-fucose was detected on EGF34 of Fibrillin-1 and Fibrillin-2 (FBN1/2) within a noncanonical POFUT1 motif. Analysis of other EGF domains containing this motif in recombinant FBN1/2 expressed in HEK293T cells identified more O-fucosylated sites. An O-fucose site was also identified in the N-terminus of Agrin. Sequence analysis indicated the presence of an unannotated EGF domain spanning the end of Kazal-like 1 and overlapping the first half of Kazal-like 2. Manual inspection of the N-terminal region identified eight potential EGF domains, two of which contain predicted POFUT1 sites. These sites are currently being validated using recombinant Agrin expressed in HEK293T cells. These data were obtained from a 10 mL input of secreted media of HEK293F cells. We expect to identify more targets with a larger input. We believe DidFAT will provide a much deeper coverage of O-fucose proteome and discover novel targets to study. This work was supported by NIH grant R35GM148433.

(PT-002) Streamlining the use of human lectins for immunological assays and immunostaining

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Human lectins play essential roles in linking the human glycome to physiological responses. However, our understanding of the interaction network between lectins and their target glycans in the cellular environment is limited due to the lack of a comprehensive collection of human lectins. To address this challenge, we at GlycoGenetics aim to establish a commercial source of recombinant human lectins. Historically, the lack of a comprehensive collection of recombinant human lectins has imposed significant challenges in investigating their biological activities such as immune cell regulation and in standardizing their use in general laboratory applications. To address these limitations, we evaluated the biological activity of a library of recombinant lectins on Jurkat T-cell populations. Critically, we expanded the application of these lectins beyond functional assays into high-precision molecular tools. By defining these functional signatures and validating their use in imaging and proteomics, we establish a framework for using human lectins as high-affinity probes essential for understanding T-cell biology and discovering novel biomarkers in complex disease states.

(PT-003) A tale of two insects: N-glycomics of the fruit fly and the silkworm

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There are millions of insect species, but only a handful of insect N-glycomes. Traditionally, insects (as other invertebrates) were considered to have simple N-glycomes with mainly oligomannosidic and paucimannosidic glycans, the latter modified by up to two core fucose residues. Later, glucuronylated and LacdiNAc motifs were found, but in the last decade we have discovered a vast range of complex anionic and zwitterionic glycan structures. Probably most work on insects, not just their glycans, has centred on the Diptera and the Lepidoptera. Our most recent studies on these two groups have been on Drosophila melanogaster, the model 'fly', and Bombyx mori, the silkworm. Although some features are shared (difucosylation, glucuronylation and mannose/fucose sulphation), the two organisms have contrasting numbers and decorations of their antennae. While the fruit fly has N-glycans with maximally three antennae and phosphoethanolamine as a zwitterionic modification, the silkworm has maximally two antennae and phosphorylcholine as the zwitterion. Furthermore, the antennae in the fruit fly are based on Hex1HexNAc1-2HexA1 motifs, never with antennal fucose. In contrast, the silkworm has not just Hex1HexNAc1HexA1 single and repeating units as 'normal' antennae, but also various modifications attached via antennal fucose residues, whether XylHexA or other HexA-containing motifs. The functions of these contrasting modifications are unclear, but the binding of the Bombyx mori nucleopolyhedrovirus to silkworm proteins is inhibited by choline phosphate and by C-reactive protein; glycan array data demonstrate binding of C-reactive protein to silkworm N-glycans and glycoproteomic data show which proteins carry phosphorylcholine-modified N-glycans. Overall, each insect species analysed to date has its own N-glycomic signature; analyses of further species are nevertheless necessary to establish a glycophylogeny.

(P-001) Functional Glycomics for Vaccines: A novel dual Enzyme Strategy for Structural Verification of Anti Pseudomonas aeruginosa Bioconjugates

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Glycoconjugate vaccines are an established antibacterial modality, and in vivo–conjugated glycoconjugates (bioconjugates) produced in glycoengineered Escherichia coli enable site specific attachment of polysaccharides to protein carriers. We describe an enzyme enabled released glycan workflow to support Pseudomonas aeruginosa bioconjugate vaccine that targets two surface virulence factors, the polysaccharide Psl and the protein PcrV. In this vaccine, PcrV is engineered to present defined O glycosylation sites, and Psl is attached at those sites via O linkages. Traditional approaches for released glycan analysis—PNGase F for N glycans and base catalyzed β elimination or oxidative chemistries for O glycans—are incompatible with this candidate due to its long polysaccharide chains and O glycosylation. To enable structural elucidation and monitoring of Psl repeating units and acyl motifs, we identified two glycan modifying enzymes from P. aeruginosa, Thioesterase A (TesA) and a Psl specific glycosidase (Pslg) and applied them as biochemical tools to trim and release analyzable fragments: TesA removes fatty acid modifications from Psl, and Pslg cleaves the polymer to liberate Psl repeating units. Released glycans and fatty acids were fluorescently labeled and analyzed by HPLC FLR and LC MS, defining the Psl repeating unit structure and revealing acyl chain heterogeneity. Deployed across multiple manufacturing batches, this characterization method enabled structural confirmation, batch to batch comparability, and correlation of degree of acylation with in vitro potency for the P. aeruginosa bioconjugate vaccine candidate.

(P-002) Multiplexed Monitoring of Recombinant Protein Glycosylation Patterns

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Accurate monitoring of glycosylation remains a major challenge in the development and manufacture of therapeutic glycoproteins. GlycoSense™, a microsphere-based multiplex lectin array platform, enables rapid near real-time profiling of glycosylation patterns without the need for time-intensive mass spectrometry workflows. Here, we demonstrate expanded applications of GlycoSense™ for monitoring recombinant glycoprotein production and culture-dependent glycosylation changes in mammalian expression systems. Using recombinant erythropoietin (EPO) as a model glycoprotein, we compared glycosylation profiles across different culture conditions including temperature and media supplementation. Lowering culture temperature from 37°C to 32°C delayed peak glycosylation and resulted in increased α2,3-sialylation as measured by MAL I binding. Similarly, supplementation with tryptone altered glycosylation kinetics, shifting peak N-glycosylation and α2,3-sialylation to later time points during expression. To further expand platform utility, we incorporated a novel N-GlyFind™ reagent into the GlycoSense™ workflow to provide a broad measure of total N-linked glycosylation alongside terminal glycan feature analysis. Together, these results demonstrate the utility of GlycoSense™ as a flexible platform for rapid monitoring of glycoprotein quality attributes and process-dependent glycosylation changes during biologics production.Supported by NIH grants R44GM153145 and R44OD035390.

(P-003) Systematic evaluation of enrichment strategies for the analysis of intact N-glycopeptides via mass spectrometry

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Intact N-glycopeptides are challenging to characterize by mass spectrometry-based proteomics. They are relatively rare in a complex biological sample, and their precursor ions are split across many glycan compositions, diluting their signal. Preparative enrichment is therefore standard practice, yet enrichment techniques have not been systematically compared across diverse sample matrices, leaving the intact glycoproteomics field without a common frame of reference. Using triplicate processing of pooled reference materials from two cell lines, three mouse organs, and cerebrospinal fluid from patients with central nervous system lymphoma, we benchmarked nine commonly used enrichment techniques – four lectin-based conditions, four solid phase extraction resins, and phenylboronic acid boronate-affinity enrichment. The mannose-binding lectin, ConA, provided the strongest single enrichment performance across nearly all evaluated metrics, despite its bias towards High Mannose glycans. Pairing ConA with a mixed-mode anion-exchange solid-phase extraction technique expanded Complex glycan coverage. By integrating cell-surface-capture data, we identified pronounced glycosite heterogeneity between tumorigenic and non-tumorigenic cell lines on a surface-exposed N-glycosite of the therapeutically relevant antibody-drug-conjugate target mesothelin. We further demonstrate tissue-dependent microheterogeneity and characterize microheterogeneity at N200 of SGCE, a proposed biomarker of treatment response in central nervous system lymphoma. Together with analyses of protocol cost and time, these results provide practical, evidence-based guidance for enrichment strategy selection in intact N-glycoproteomics experiments.

(P-004) Towards gaining glycan topology understanding from glycoproteomics experiments

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When glycoproteomics experiments are performed using LC-MS/MS, the chromatographic retention time and fragmentation patterns contain information about glycan structure. Yet, most glycoproteomics software primarily focus on glycan composition, in part because existing approaches evolved from classical proteomics workflows that treat glycans as post-translational mass modifications. Consequently, much of the structural information contained within glycopeptide spectra remains underutilized. To address this limitation, we developed an integrated experimental and computational workflow aimed at extracting glycan topology from LC-MS/MS data. The computational framework consists of three complementary, Python-based open-source tools integrated with a graphical user interface (GUI) in a toolkit called GlycoStudio: i) pyDrawGlycan is fully compatible with the Symbol Nomenclature for Glycans (SNFG) and renders glycans and glycopeptides, including their fragmentation patterns; ii) GlycoSpectrumAnnotation

enables single-spectrum annotation of glycan and glycopeptide MSn data based on glycosidic and peptide fragmentation ions, oxonium ions, neutral losses, internal fragments, and cross-ring fragmentation in both positive and negative ion modes. iii) pyGlycoPAT enables large-scale analysis of N-glycoproteomics datasets and incorporates algorithms to extract glycan topology information from experimental spectra. For experimental validation, we optimized glycopeptide enrichment and fragmentation workflows using human plasma samples. HILIC enrichment was coupled with complementary acquisition strategies on the Orbitrap Astral (stepped-energy HCD) and Fusion Lumos (HCD-triggered EThcD) platforms. The workflow was further extended to TMT-labeled glycopeptides, where fragmentation conditions must simultaneously support reporter-ion generation, peptide sequencing, and glycan structural characterization. Together, the paper illustrates how complementary fragmentation strategies, coupled with glycan-specific computational analysis, can provide a more complete topological description of the human plasma glycoproteome.

(P-005) Cysteine-directed enrichment maps in vivo Protein O-glucosyltransferase 2 and 3 substrates in mouse lung

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Protein O-glucosyltransferase 2 and 3 (POGLUT 2 and 3) catalyze O-glucose addition to serine within the consensus sequence C3-x-x-x-x-x-S-x-x-C4 of properly folded epidermal growth factor (EGF)-like repeats. In Fibrillin-1 (FBN1) and FBN2, approximately half of the 47 EGF repeats are modified by O-glucose. Fibrillin microfibrils are critical for elastic fiber assembly, tissue development, growth factor regulation in the extracellular matrix. Poglut2/3 double knock out (DKO) mice exhibit neonatal lethality, and rare survivors have digits 2-3 syndactyly, cardiopulmonary, and ocular defects resembling fibrillin/elastin mutant phenotype. There are many more proteins with EGF repeats containing the consensus sequence that may contribute to DKO abnormalities, emphasizing the need to define proteins with O-glucose modifications through targeted enrichment. To enrich O-glucosylated peptides, we used a selective thiol-labeling strategy using the sulfhydryl-reactive alkylating reagent called “Cysteine-specific Phosphonate Adaptable Tag (6C-CysPAT)”. Since each EGF repeat contains six conserved cysteines linked by disulfide bond, enrichment of cysteine-containing peptides enables co-enrichment of O-glucosylated peptides. The workflow was optimized using the N-terminal of human FBN1 expressed in wild-type (WT) HEK293T suspension cells. Proteins were reduced, alkylated using 30 mM CysPAT, excess reagent was removed, followed by C18, and labeled peptides were enriched by Immobilized Metal Affinity Chromatography. Data were acquired on a Q-Exactive Plus for method development and a timsTOF Flex for complex biological samples and glycopeptides were identified using Byonic v5.8.6. The optimized workflow achieved >98% CysPAT labeling efficiency and enriched O-hexose modified peptides spanning hFBN1 EGF repeats 6,8,9,10,12,13,15,17 and 21-23, along with the known O-fucosylated EGF2 peptide, from purified protein, secretion media, and cell lysate. Application of this strategy to lungs from Poglut2/3 DKO and double Heterozygote (control) mice identified several previously uncharacterized in vivo POGLUT2/3 substrates, including nephronectin EGF2_S110, EGF4_S192, and EGF5_S237; hemicentin-1 EGF2_S5169; complement C1q receptor EGF5_S446; and LRP1 EGF4_S825. Nephronectin O-glucosylation was validated in WT, Poglut2/3 DKO, Poglut2 KO, and Poglut3 KO HEK293T cells, demonstrating high stoichiometric O-glucose occupancy >93% at EGF2 and POGLUT2-specific preference for EGF4. This workflow is being extended to map O-fucose, EOGT-mediated O-GlcNAc, and non-canonical O-hexose sites across the same sample sets and to investigate how O-glucosylation regulates protein secretion. Collectively, this thiol-directed enrichment strategy enables robust mapping of O-glucose sites and in vivo POGLUT2/3 substrates, that will advance our understanding of how O-glucosylation regulates protein function and contributes to connective tissue disorders. Supported by NIH grant HL161094.

(P-006) GlycoMsHelper: A High-Confidence Glycomic MS Data Interpretation Workflow

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Glycosylation is a fundamental post-translational and lipid modification essential to diverse cellular processes. While mass

spectrometry (MS) has become the primary platform for glycomic analysis, large-scale interpretation of glycomic MS data remains heavily reliant on expert manual annotation, representing a major bottleneck in the field. Key challenges in automating glycomic data interpretation process include effective spectral denoising and reliable identification of glycan-derived MS/MS (MS2) spectrum. Here, we present GlycoMsHelper, a flexible R-based workflow with a user-friendly graphical interface (GUI) designed to streamline positive mode glycomic MS data composition-level analysis. GlycoMsHelper integrates multiple denoising strategies with logical expression-based filtering method to confidently isolate glycan-derived MS2 spectra from large numbers of acquired spectra. Filtered candidate spectra are then matched against glycan libraries constructed using curated biosynthetic constraints and generate traceable outputs for downstream validation. By defining appropriate parameters, the framework can be applied to diverse glycan classes, including N-glycans, O-glycans, and glycosphingolipids (GSLs). Because each module operates independently, produces standardized outputs, and is supported by a GUI, GlycoMsHelper can be readily integrated into existing pipelines as a practical complement to currently available analysis tools. This tool is particularly valuable for exploratory analysis of newly acquired or previously uncharacterized datasets where established reference libraries are unavailable, offering an accessible and practical solution for semi-automated glycomic MS data interpretation.

(P-007) From Days to Hours: A Unified One-Flow Strategy for Rapid N- and O-Glycan Release in High-Throughput Glycomics

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Glycoproteins and their conjugated N- and O-linked glycans serve as vital biomarkers for biological characterization and disease diagnostics, and therapeutic efficacy. These glycans influence key biological processes such as protein stability, cell signaling, and immune recognition. However, existing workflows for comprehensive glycan profiling remain slow, labor-intensive, and prone to inefficiencies. Conventional methods typically require sequential, multi-day steps: overnight enzymatic release of N-glycans (PNGase F), followed by a separate overnight chemical β-elimination for O-glycans, along with repeated molecular weight cut-off (MWCO) filtration, desalting, and cleanup steps. These cumbersome procedures not only extend turnaround times but also risk sample loss and introduce contaminants that compromise downstream derivatization chemistries such as permethylation.Here, we introduce the Fast One-Flow method for the simultaneous release of both N- and O-glycans within a single workday. Utilizing an optimized unified buffer and processing system, this approach eliminates the need for intermediate cleanup or enrichment steps while maintaining direct compatibility with downstream analyses. We validated the robustness of this workflow through successful permethylation a highly impurity-sensitive derivatization technique which yielded enhanced ionization efficiency, stabilization of labile sialic acid moieties, and seamless integration with LC-MS platforms.By condensing sample preparation from multiple days to less than 24 hours, the Fast One-Flow method offers a cost-effective, scalable, and high-throughput solution for comprehensive glycomics analysis across complex biological samples, significantly advancing large-cohort studies and clinical translation.

(P-008) The Emory Glycomics and Molecular Interactions Core (EGMIC): Advanced Capabilities in Mass Spectrometry, Glycan Profiling, and Biomolecular Interactions

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The Emory Glycomics and Molecular Interactions Core (EGMIC) provides cutting-edge mass spectrometry instrumentation, glycan microarrays, and molecular interaction technologies to advance biomedical research. Our advanced liquid chromatography–mass spectrometry (LC–MS) suite features high-performance systems, including the Agilent 6560 Ion Mobility Q-TOF, Agilent 6545XT AdvanceBio Q-TOF, Thermo TSQ Altis QQQ, and Thermo Orbitrap ID-X. To further enhance discovery capabilities, EGMIC is currently acquiring the Bruker timsOmni LC–MS platform, which will significantly enhance MS and MS^n sensitivity and performance. Beyond mass spectrometry, the core provides state-of-the-art platforms for analyzing glycan functions and biomolecular interactions. Key instrumentation includes the Innopsys InnoScan microarray slide scanner for high-density glycan interaction profiling and the Malvern Panalytical MicroCal Auto-iTC200 for direct, label-free measurements of thermodynamic parameters and binding affinities. Additionally, the recently acquired Cytiva Biacore 1K system enables automated surface plasmon resonance (SPR) profiling and real-time kinetic analysis. EGMIC also provides specialized services for N- and O-glycan mass profiling and custom shotgun glycan microarray development. The core operates under a flexible, user-centric service model,

offering both independent instrument training for trainees and fellows and full-service sample analysis by expert staff. We support investigators through comprehensive experimental consultation, technical training, and hands-on operational assistance across all platforms.

(P-009) N-GlyRichTM and SiaRichTM: Affinity resins for specific enrichment of N-glycosylated and sialylated glycoproteins and glycopeptides

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SiaFindTM α2,3-Specific Lectenz®, Pan-Specific Lectenz®, and α2,6-Specific Reagent are a collection of recombinant proteins engineered for highly specific binding to different sialic acid linkages of sialylated glycoconjugates. SiaFindTM α2,3- and Pan-Specific Lectenz® selectively recognize α2,3-siaylated conjugates and linkage-independent sialylated conjugates, respectively. SiaFindTM α2,6-Specific Reagent exclusively recognizes α2,6-sialylated conjugates. In parallel, we have also developed N-GlyFindTM, an affinity reagent specifically recognizing the ManGlcNAc2-Asn core present in all N-glycans. SiaFindTM and N-GlyFindTM reagents have already been used in various applications including Western blotting, ELISA, flow cytometry, and immunohistochemistry. To use these reagents for affinity chromatography we have recently developed SiaRichTM and N-GlyRichTM resins for the enrichment of intact glycoproteins and glycopeptides. Prepared in a benchtop-ready format, SiaFindTM and N-GlyFindTM proteins were coupled to an agarose resin, rigorously tested and validated for optimal separation of target glycoconjugates. Each SiaRichTM resin retained the binding specificity of its coupled affinity reagent, as demonstrated by the resins' selective enrichment or exclusion of natural glycoproteins, such as fetuin, asialofetuin, as well as a suite of our Asn-linked neoglycoproteins. The N-GlyRichTM resin precisely separates N-glycoproteins and non-glycosylated proteins. It has the capacity of enriching N-glycopeptides from complex samples for mass spectrometry applications. Supported by NIH grants R44OD024964 and R44OD035390.

(P-010) A Rapid and High-Throughput Biotechnology Quantifies Major Glycoforms of Human IgG1 mAb Drugs

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Human IgG1 monoclonal antibody (mAb) drugs are widely manufactured using mammalian cells and contain three major glycoforms on asparagine residue at position 297 (Asn297) in their Fc region: fucosylated, afucosylated, and high-mannose glycans. The N-glycans at Asn297 directly impact IgG1 mAb efficacy through effector functions and half-life. Thus, N-glycosylation is a critical quality attribute (CQA) for IgG1 mAb therapeutics, and needs to be controlled during manufacturing and release to ensure lot-to-lot consistency and demonstrate similarity for IgG1 mAbs and biosimilars. In this study, we generate two mouse mAbs, mAb-NG and mAb-G, that specifically recognize nonglycosylated and glycosylated human IgG1, respectively. Using the mAb-G, we establish a technology in a biolayer interferometry (BLI) format with high-throughput potential that rapidly quantifies the relative amount (%) of each glycoform of IgG1 mAbs, either purified or in harvest media. This biotechnology can accelerate cell line and biomanufacturing process development, and potentially serve as a component of process analytical technology (PAT) for IgG1 mAb drugs.

(P-011) Evaluating the Efficiency of Ionic Liquid Matrices as Performance Enhancers for High-Throughput MALDI-MS Glycan Analysis

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Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is widely used for the characterization of glycans because of its sensitivity, speed, and ability to analyze structurally diverse oligosaccharides. However, conventional crystalline matrices can result in heterogeneous analyte distribution, variable crystallization, matrix-associated background, and inconsistent ionization, potentially affecting the reproducibility of glycan profiling. Ionic liquids (ILs) and ionic liquid matrices (ILMs) are found to offer an alternative approach because of their non-crystalline nature and provides improved sample homogeneity and more consistent analyte incorporation. But their relative performance across different glycan classes and derivatization states remains incompletely characterized.In this study, we evaluate the performance of ILs and ILMs for the MALDI-MS characterization of native and permethylated glycans and compare their analytical performance with commonly used conventional matrices. A representative panel of neutral and acidic oligosaccharides derived from various complex glycan sources was analyzed using different IL matrix formulations under optimized MALDI conditions. Matrix performance was evaluated based on ionization efficiency, signal intensity, background interference, reproducibility, and preservation of labile glycan structures. MS/MS analyses were additionally performed to assess whether the use of IL matrices influences glycosidic-bond fragmentation and the generation of structurally informative fragment ions. Finally, the suitability of these matrices for high throughput glycomics workflows was investigated.In most cases, the morphology of the matrix layer was observed to be uniform, unlike that of conventional matrices. This resulted in highly homogeneous sample distribution throughout the spot, allowing reproducible and consistent mass spectra to be obtained without spot-to-spot variations in signal. The results demonstrate the potential of ILs and ILMs to improve the homogeneity and reproducibility of MALDI glycan analysis. Our findings provide practical guidance for matrix selection in glycomics workflows, demonstrating the role of ILMs for native, underivatized, and labile (sialylated/sulfated) glycan classes, along with the observations for the permethylated samples. The work supports a rational, sample-class-specific approach to matrix selection for MALDI-based glycan characterization.

(P-012) Strategies for Comprehensive Mass Spectrometry Characterization of Lipid A in Gram-negative Pathogens

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Lipid A is the membrane-anchoring region of lipopolysaccharide (LPS) and plays an important role in bacterial membrane stability and host immune recognition. Structural variation in lipid A, including differences in acylation pattern, phosphorylation, or modification with carbohydrate moieties, can influence bacterial physiology and interactions with the host. In addition to chemical and NMR analyses, extensive mass spectrometry methods have become important tools for detailed characterization of lipid A structure. In this study, we compared different mass spectrometry fragmentation methods for structural characterization of lipid A with chemical methods to provide complementary information for structural characterization of Lipid A from Escherichia coli and Fusobacterium nucleatum isolates. Lipid A was first isolated and purified from each investigated pathogen, followed by chemical analyses by gas chromatography-mass spectrometry (GC-MS) to define the molar distribution of fatty acids and carbohydrates constituting lipid A. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and nano-spray ionization mass spectrometry (NSI-MS) were used to examine the molecular ion profiles of intact lipid A species. Tandem mass spectrometry in High Resolution Orbitrap Ascend mass spectrometer using 213nm ultraviolet photodissociation (UVPD), higher-energy collisional dissociation (HCD), and collision-induced dissociation (CID) fragmentation methods were then used to determine the acyl-chain distribution, backbone substitution, and phosphate-containing regions. Together, these approaches provide a more complete understanding of lipid A structural diversity and help establish an analytical framework for future studies of lipid A modifications to aid in understanding the biological role.

(P-013) Robust One-Step Method for Sulfation Level Characterization of Sulfated Heparin Oligosaccharides Using Trifluorodiazoethane

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Glycosaminoglycan (GAG) analysis remains a significant analytical challenge due to structural complexity, heterogeneity, and high degree of sulfation of these molecules. Accurate characterization of sulfation level is particularly difficult by conventional LC-MS because sulfate groups are labile during mass spectrometric analysis, resulting in sulfate loss. Although several methods have been developed to transform highly anionic sulfates into hydrophobic esters, most require multiple steps and fairly complex protocols. We present a one-step chemical derivatization strategy using 2,2,2-trifluorodiazoethane (TFD) to convert sulfate groups into stable trifluoroethyl sulfate esters, that increase hydrophobicity, improve chromatographic resolution, and enable MS detectability while preserving the native sulfation levels and structure. The extracted-ion chromatograms (EICs) of typical LC-MS runs provide direct detection of intact peresterified species, enabling assessment of sulfation state without specialized instrumentation or extensive data analysis. Briefly, sodiated GAGs were treated with an ethereal solution of TFD in acetonitrile in the presence of citric acid, followed by SpeedVac removal of the reagent, reconstitution in acetonitrile/water, LC–MS injection, and ESI–MS analysis, which confirmed the peresterified species and their corresponding m/z values. Proof-of-concept studies were performed with heparin disaccharide, sucrose octasulfate and fondaparinux. The one-step reaction products could be directly analyzed by HR-MS to yield the accurate mass of the parent sulfated species, which is a key structural parameter of use in development of chemistry, manufacturing, and controls (CMC) documentation, while also assisting with quality control and batch-to-batch variability of sulfated oligosaccharide products. The method may also aid sequencing of oligosaccharide chains using LC-MS/MS approaches.

(P-014) 213 nm UV photodissociation for high-resolution structural analysis of permethylated glycans

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Ultraviolet photodissociation (UVPD) continues to be a powerful technique for the structural characterization of biomolecules, including proteins, lipids, nucleic acids, and carbohydrates. Although collision-induced dissociation (CID) is commonly used for glycan analysis, UVPD offers an important advantage by generating cross-ring fragment ions that can provide detailed information about glycan linkages and structural features at MS2 level. Previous studies have shown that 157nm and 193nm UVPD generates abundant cross-ring and diagnostic fragments, enabling glycan isomer differentiation. However, these studies used in-house UV laser setup and low-resolution ion-trap instruments. In this study, we explored capability of 213 nm laser-induced UVPD coupled with high-resolution Orbitrap mass spectrometry for structural characterization of permethylated human milk oligosaccharides (HMOs) and glycans. This approach produced distinct fragmentation patterns for permethylated lacto-N-neotetraose (LNnT) and lacto-N-fucopentaoses (LNFPs), showing distinct cross-ring fragments enabling differentiation of their isomers. In comparison to CID, 213nm UVPD generated numerous cross-ring fragments from permethylated disialylated mucin O-glycan, showing distinct fragments that differentiated α2,3- and α2,6-linked sialic acids and confirmed their coexistence. We also applied this method to complex biological samples obtained from healthy and glioblastoma human brain tissues for disialylated O-glycan. This produced cross-ring fragments consistent with those observed from mucin standard, demonstrating its applicability to biologically relevant samples. Furthermore, the approach enabled the detection of characteristic fragment ions associated with bisecting N-acetylglucosamine (GlcNAc) in human immunoglobulin G (IgG) N-glycans directly at the MS2 level. Overall, these results demonstrate that 213 nm UVPD combined with high-resolution Orbitrap mass spectrometry is a promising approach for glycan structural characterization with improved mass accuracy. This strategy has the potential to advance comprehensive structural glycomics and improve the characterization of complex glycan alterations in biological and disease-associated samples.

(P-015) Complementary Distribution of Glycoconjugates Revealed by Multi-Enzyme MALDI Mass Spectrometry Imaging

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Glycoconjugates play crucial roles in cancer biology, particularly in tumor progression and invasion. However, their spatial distribution and potential functions in tumor microenvironments remain poorly understood due to technical limitations in analyzing complex glycan structures in situ. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) has emerged as a powerful tool for spatial analysis of biomolecules, but its application to glycoconjugate imaging has been limited by the complexity of glycan structures. Here, we present a combination of specific enzymatic treatments with MALDI-MSI that offers a novel approach to overcome these limitations, enabling detailed spatial mapping of different glycoconjugate classes. This technique can unravel biochemical implications of tumor growth and help in biomarker discovery and margin detection studies. Chondroitinase (A, B, or C) treatment breaks different types of CS to smaller disaccharide units that are more easily ionized in the MALDI source and enables us to expose a statistically significant enrichment of chondroitin sulfate disaccharides within tumor regions compared to surrounding healthy tissue. Employing complementary enzymatic digestions in addition to the use of higher resolution MSI can provide sharp visualizations of different tissue types. The results show satisfactory compliance with histopathology results. Next, N-glycan imaging demonstrates different distribution pattern for different N-glycans in the mouse brain tissue. The spatial patterns observed through MALDI-MSI shows satisfactory correlation with H&E staining, confirming the precise localization of different glycan species is achievable and notably comparable to histopathological experiments that are more standardized. Additionally, enzyme-facilitated MALDI-MSI of glycoconjugates provides valuable biochemical insights. Imaging data of glycoconjugate distributions of tumor and non-tumor regions, demonstrates distinct boundary patterns that could potentially serve as molecular markers for tumor margins.

(P-016) *O*-Glycosylation as Regulator of Amyloid-β and Tau Pathology in Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the deterioration of neuronal structure and function. Despite its growing societal and healthcare burden, currently available therapies provide limited disease-modifying benefit. AD is characterized by two hallmark protein aggregates: extracellular amyloid-β (Aβ) plaques derived from amyloid precursor protein (APP) processing and intracellular neurofibrillary tangles composed of aggregated tau. Although the amyloid cascade hypothesis has long guided therapeutic development, the limited success of Aβ-targeted therapies has highlighted the need to better understand the molecular mechanisms governing both Aβ and tau pathology. Post-translational modifications (PTMs) are increasingly recognized as key regulators of protein homeostasis in AD. APP processing by α-, β-, and γ-secretases controls Aβ production, while emerging evidence suggests that mucin-type *O*-glycosylation modulates APP processing and aggregation behavior. Similarly, tau conformation and aggregation are regulated by PTMs, particularly phosphorylation and *O*-GlcNAcylation, which have been implicated in the formation or prevention of pathogenic tau assemblies. To investigate the influence of PTMs on AD-associated protein aggregation, we synthesized APP-derived glycopeptides and tau peptide fragments using solid-phase peptide synthesis. APP models spanning the Aβ(1-23) region contained native or Swedish-mutated sequences and site-specific *O*-GalNAc modifications at Thr663, Ser667, and/or Tyr681. Tau fragments containing the PHF-1 epitope were modified with either phosphorylation or *O*-GlcNAcylation at Ser396 and Ser400. Structural and functional effects were evaluated using circular dichroism spectroscopy, HPLC, mass spectrometry, Thioflavin T fluorescence assays, and transmission electron microscopy. PTM identity, site occupancy, and glycan density significantly influenced protein conformation, proteolytic processing, and aggregation behavior. In APP models, increased glycan valency promoted β-turn-rich conformations and altered secretase activity, whereas the Swedish mutation enhanced β-secretase cleavage. Glycosylation also modulated Aβ40 aggregation, with multivalent glycopeptides delaying fibrillization and favoring oligomeric species, while Ser667 glycosylation promoted fibril formation. In tau models, phosphorylation enhanced

fibrillization and aggregate maturation, whereas *O*-GlcNAcylation attenuated fibril formation and altered aggregation during both independent assembly and co-incubation with Aβ40. Collectively, these findings demonstrate that site-specific glycosylation and phosphorylation serve as critical regulators of APP processing, Aβ aggregation, and tau fibrillization. These findings highlight PTM crosstalk as a key determinant of AD-associated protein homeostasis and support glycosylation-mediated pathways as promising targets for disease-modifying therapies.

(P-017) Machine Learning-Assisted Surface-Enhanced Raman Spectroscopy for Quantitative Glycan Analysis

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Glycosylation patterns encode critical information about cellular state and disease, yet monitoring them accurately and frequently remains a persistent analytical challenge. To overcome these challenges, we previously developed a novel sensing platform that combines a boronic acid (BA) receptor array, surface-enhanced Raman spectroscopy (SERS), and a machine learning (ML) model to enable rapid glycan analysis. BAs capture glycans from complex biological samples by reacting with the hydroxyl groups on glycan molecules. To recognize structural differences, the sensor monitors the full Raman spectrum, which provides a specific molecular fingerprint. By analyzing these unique Raman spectra, the structures of the glycans can be identified. However, training machine learning (ML) models to decode these complex spectra traditionally relies on time-consuming, expensive dataset collection. To overcome this bottleneck, we report an end-to-end analysis protocol for simultaneous quantification of glycan mixtures. Our workflow integrates: (1) a computationally constructed Raman spectral library to bypass experimental data acquisition; (2) a spectral correction model that bridges discrepancies between simulated and experimental spectra using minimal training data; and (3) an uncertainty-aware prediction model to accurately quantify glycan concentrations. By combining scalable computational training with experimental validation on physical glycan mixtures, this framework drastically reduces the need for exhaustive experimental training datasets. Coupled with emerging portable SERS hardware, this platform offers a fast, scalable solution for point-of-care glycan analysis.

TOPIC 2: AI, Informatics, and Computational Glycoscience

(KEY2-001) Ancient MUC1 repeat expansion links mucus barrier defense to pleiotropic tradeoffs

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Mucin-1 (MUC1) is a transmembrane glycoprotein that forms the mucus barrier and contains a polymorphic VNTR modulating protein length and glycosylation. Using 822 haplotype-resolved long-read assemblies as a pangenome to impute ancient and archaic genomes , we reconstruct the evolutionary and functional history of MUC1 repeat variation. Alleles segregate into four length groups—short, medium, long and extreme. MUC1-long is specific to humans, and rose markedly across Europe during the agricultural transition. MUC1-long was also found to be associated with several adverse phenotypes, including kidney and pregnancy traits, whereas MUC1-short was linked to higher erythrocyte measures and convergent enrichment in Tibetan and Andean highlanders. Together, these results support a model in which the MUC1 repeat expansion enhanced mucus-barrier defense amid changing Holocene zoonotic pressures, while generating population-specific physiological tradeoffs. More broadly, our findings reveal VNTR copy number as a malleable substrate of pleiotropic human adaptation and evolution of the mucus barrier.

(P-018) Protein language model-based prediction of heparan sulfate binding proteins with HSBPFinder

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Heparan sulfates (HS) are ubiquitously expressed glycans that interact with HS binding proteins (HSBPs) to mediate growth factor and cytokine signaling, cell adhesion, and extracellular matrix formation. Deletion of HS synthesis genes is embryonically lethal, and dysregulation of HS and HSBPs is implicated in the pathogenesis of cancer, Alzheimer's disease, sepsis, and obesity. HS and HSBPs are therefore critical regulators of cell and tissue homeostasis, and discovery of new HSBPs could potentially inform development of novel therapeutics for several diseases. While over 500 HSBPs have been identified, this list is heavily biased toward secreted extracellular proteins, which comprise only ~15% of the human proteome. To map HSBPs across the full human proteome, we developed HSBPFinder. HSBPFinder leverages AI-based protein language modeling to predict the likelihood that a protein binds HS. To train the model, we curated a dataset called HSBPatches, which contains 298 HSBPs annotated with residues shown to be essential for HS binding. Receiver operator characteristic (ROC) curve analyses indicated that HSBPFinder achieved an AUROC of 0.89 on a held-out validation set of known HSBPs, outperforming two state-of-the-art glycan binding prediction models (PiCAP: AUROC=0.81 and Pesto-Carbs: AUROC=0.52). HSBPFinder nominated several novel HSBPs with little sequence homology to known HSBPs. Additionally, HSBPFinder accurately classified several known bacterial HSBPs, indicating that this model performs well for both eukaryotic and prokaryotic organisms. These results establish HSBPFinder as a novel tool for the identification of HSBPs across the tree of life.

(P-019) A Learned 3D Representation of Glycan Conformational Flexibility

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Glycan recognition depends on the conformational ensemble a glycan samples in solution, not on any single static structure, yet most computational representations reduce glycans to sequence strings or 2D linkage graphs, discarding this entirely. Recent work has shown that incorporating glycan 3D structural information derived from molecular dynamics can improve prediction of glycan-protein binding, but existing approaches rely on a small set of derived, per-residue summary statistics computed only for glycans with existing simulation data. We present a machine learning architecture designed to predict a full, spatially resolved representation of glycan conformational behavior directly from its 3D structure, trained against population-weighted molecular dynamics ensembles obtained from the GlycoShape database. The model uses a graph neural network architecture, encoding both the ensemble-derived position of each atom and how much, and in what pattern, it moves around that position, captured directly from the weighted conformer ensemble rather than assumed form a single structure or inferred from sequence alone. This ensemble-aware encoding allows the predicted representation to reflect a glycan's real conformational behavior rather than a single static snapshot. We are also investigating whether the model can be trained to predict this representation for glycans with no existing simulation data, using structure-only inputs alongside the ensemble-derived training signal, which would extend the approach beyond the fixed set of computationally simulated glycans currently available.Beyond atomic position and flexibility, the design of this representation accommodates additional physiochemical properties, including those relevant to water-mediated hydrogen bonding in glycan-lectin recognition. We are also building a second-stage model that uses this representation to predict protein-glycan binding, which we are evaluating against existing sequence- and graph-based approaches. More broadly, a generalizable, ensemble-aware representation of glycan conformational behavior may support additional applications including glycoprotein structure prediction, interpretation of glycan array data, and mechanistic studies of protein-glycan interactions.

(P-020) Fine-tuning a Transformer Model for Glycan Structure Inference from Under-represented MS/MS Spectra

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Glycan structure annotation from tandem mass spectrometry (MS/MS) remains a bottleneck in high-throughput glycomics. Deep-learning (DL) models have recently emerged as a promising alternative to traditional library search and de novo sequencing strategies. We previously developed two transformer-based models, GlycoBERT and GlycoBART, for glycan structure prediction from MS/MS data (Abtheen et al., bioRxiv 2025, doi:10.1101/2025.07.02.662857). Both achieved high structural prediction accuracy on a held-out test set, outperforming the CNN-based model CandyCrunch trained on the same data (Urban et al., Nat. Methods 2024, 21:1206–1215). Unlike GlycoBERT and CandyCrunch, which are classifiers, GlycoBART is a generative model capable of predicting de novo structures absent from the training set, making it well suited to fine-tuning for specific applications.While these DL models perform well on spectra resembling their training data, accuracy drops sharply on out-of-distribution spectra—those whose glycan types, motifs, or acquisition conditions are poorly represented in the training set. For example, on positive-mode MS/MS spectra from a mouse N-glycome study (Helm et al., Nat. Commun. 2024, 15:9725; GlycoPOST GPST000398), overall performance fell markedly—less than 22% of the spectra were annotated by the above DL models, and prediction of N-glycans bearing the Neu5Gc motif was especially poor (<0.5%). These results reflect the scarcity of such spectra in the CandyCrunch training set, in which spectra from Neu5Gc-bearing N-glycans are rare (~3.5%) and essentially absent under positive mode.To improve inference performance for these under-represented spectra, we applied transfer learning to fine-tune GlycoBART using library-annotated spectra from two mouse tissues, with hyperparameters optimized by 5-fold cross-validation (learning rate 10-7, 90 epochs). We evaluated the fine-tuned model on the remaining 18 tissues using three metrics: (i) coverage, the relative abundance of annotated N-glycans; (ii) topological accuracy against library-based annotation; and (iii) Jaccard index (JI), measuring agreement with peak-based annotation for a given motif (e.g., Neu5Gc). Fine-tuning improved coverage from 12.7% to 63.5% and topological accuracy from 35.8% to 68.6%. Of note, Neu5Gc coverage rose from 6.3% to 61.5% and JI from 0.4% to 57.0%. These findings position GlycoBART fine-tuning as an effective and generalizable strategy for annotating under-represented glycan spectra, expanding the applications of deep learning in high-throughput glycomics.

(P-021) Glycan Composition Inference and Identifiability from Mass and Biosynthetic Constraints

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Glycans play critical roles in infection, immunity, and therapeutic antibody function. For glycans lacking comprehensive reference databases, composition assignment can still rely on manual enumeration of anticipated candidates, a process difficult to generalize and potentially overlooking unusual compositions. Existing software generally does not characterize composition ambiguity analytically or predict a priori when mass alone suffices for unique identification.We formulate composition assignment as a constrained combinatorial problem. Given an observed mass, the method solves a linear Diophantine system over user-defined residues and constraints, returning all feasible solutions while jointly considering composition, adduct, charge state, derivatization, and reducing-end chemistry. The method applies across glycan families without pre-enumerated composition or structure libraries. Against independently curated GlyCosmos structures, it recovered 325/325 high-mannose N-glycans, 569/572 sialyl Lewis x-containing glycans, and 6/7 GXM compositions. The unrecovered GXM structure contained a non-canonical xylose–xylose extension outside the biosynthetic model, a constraint violation flagging exceptional structures rather than computational errors. From raw MALDI-TOF spectra of PA-labeled tardigrade N-glycans, analyzed without the authors' assignments, the method uniquely recovered all 20 published compositions (mean residual error 0.03 Da) while independently recovering the derivatization offset. In Orbitrap spectra of permethylated Cryptococcus neoformans N-glycans, it recovered 36 high-mannose compositions across four strains (mean residual error 0.006 Da). The implementation is publicly available. We derive a quantitative account of composition ambiguity. The number of mass-consistent compositions is governed by an integer denumerant predicting linear growth with mass tolerance and polynomial growth with glycan mass. Biosynthetic constraints reshape this feasible space and can sharply reduce degeneracy. We further define a family-specific uniqueness boundary, $M^*(\delta)$, the mass above which composition is generically no longer uniquely determined at a given tolerance. Thus, we quantify how measurement accuracy and biosynthetic constraints jointly

determine whether mass alone can resolve glycan composition.The framework extends auditable composition inference to incompletely catalogued glycans while establishing the capabilities and quantitative limits of mass-based identification. It predicts when mass alone is sufficient or higher mass accuracy, tandem MS, or additional structural data may be required.

(P-022) Are N-Linked glycans intrinsically disordered?

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The covalent attachment of oligosaccharides to asparagine side chains on protein surfaces (N-linked glycosylation) is a ubiquitous modification that is critical to protein stability and function. Experimental 3D structures of glycoproteins in which the N-linked glycans are well resolved are rare, due to both the presumed flexibility of the N-linked glycan and to glycan microheterogeneity. To surmount these limitations, computational modeling is often applied to glycoproteins, particularly to generate an ensemble of 3D shapes for the N-linked glycans. While the number of glycoprotein modelling tools continues to expand, the available experimental data against which the predictions can be validated remains extremely limited. Here, we present our current understanding of the dynamic properties of N-linked glycans, with a particular focus on features that impact their presentation (orientation) relative to the protein surface. We discuss the current limits of molecular dynamics (MD) simulations of glycoproteins and introduce a maximum entropy alternative that offers advantages in speed and conformational sampling. Lastly, we review the limits of experimental and theoretical studies of glycoproteins, and answer the question, “are N-linked glycans intrinsically disordered”.

(P-023) GlyGen: Advancing Glycoscience Through Intelligent Data Discovery, AI-Driven Access, and Community Data Sharing

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Glycosylation is the most common and biologically significant post-translational modification, influencing protein structure, cell signaling, development, and disease. Despite its critical role in biology, glycoscience research remains challenging due to the structural complexity of glycans and the fragmentation of glycan and glycoprotein data across many databases. GlyGen addresses these challenges by serving as a FAIR-compliant glycoscience knowledgebase that integrates glycans, glycoproteins, glycosylation enzymes, and their associated biological knowledge within a unified platform.GlyGen aggregates and harmonizes data from major international resources, including EBI, NCBI, PDB, GlyConnect, GlyTouCan, and GlyCosmos, along with direct contributions from the research community. Through standardized identifiers, cross-references, and curated annotations, GlyGen enables seamless navigation across glycans, glycan motifs, proteins, pathways, diseases, biomarkers, and publications. These interconnected datasets are freely accessible through the GlyGen web portal (https://glygen.org), REST APIs, downloadable datasets, and a SPARQL endpoint, supporting both experimental and computational research workflows.To enhance data access and discovery, GlyGen has developed an AI Query Assistant that uses large language models (LLMs) to translate natural-language questions into structured API queries. By grounding query generation and data retrieval in GlyGen resources, the system minimizes unwanted behavior and hallucinations in LLM outputs. To expand the AI-based data access strategy, GlyGen implemented a Model Context Protocol (MCP) server that enables AI agents and chatbots to directly access, query, and reason over GlyGen data, supporting natural-language exploration of glycoscience knowledge.In addition, GlyGen provides infrastructure for community-driven data deposition, enabling researchers to submit and share standardized glycomics and glycoproteomics datasets. Submitted data is publicly accessible and disseminated through GlyGen, and shared with other public resources, increasing visibility, reuse, and long-term value of the data. For a demonstration of GlyGen or to contribute data, please contact Sujeet Kulkarni (sujeet.kulkarni25@uga.edu).

(P-024) Connecting Diverse Glycan Data through Standardized Representations and Database Integration

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Glycan-related information encompasses diverse data types, including chemical structures, compositions, mass spectrometry data, three-dimensional structures, and biological annotations, which are often stored and analyzed in different databases and computational environments. To enable their cross-resource use, standardized representations and identifiers are required to describe glycan structures consistently and to establish reliable correspondences among heterogeneous data resources.We have been developing data-integration approaches based on the Web3 Unique Representation of Carbohydrate Structures (WURCS). One example is the integration of chemical structure resources such as PubChem with glycan-specific resources such as GlyTouCan. A key challenge in this integration is determining which parts of chemical structures should be recognized as glycans. To address this problem, we have developed algorithms and software for extracting glycan-like substructures from chemical structures and converting them into standardized WURCS representations. These representations can then be compared with registered glycan structures and used to establish links between chemical and glycan databases. We are also developing methods to evaluate the suitability of converted structures for database integration through syntax validation, structural comparison before and after format conversion, and filtering based on glycan-like structural features.These efforts have highlighted practical challenges in glycan data integration, including differences in structural representation, ambiguity in structural definitions, data-quality issues, and the need to define the scope of structures represented in individual resources. As a further extension, we are exploring standardized ways to describe and identify glycan three-dimensional conformations. Such representations may enable conformational information to be linked to existing glycan identifiers and other experimental and computational data resources.Here, we present these efforts as steps toward an interoperable glycan information infrastructure and discuss how standardized representations and shared identifiers can facilitate reuse of existing data, improve data quality, and support integration of chemical, structural, and biological information across glycoscience.

(P-025) KB-TOHSA: An RDF Knowledge Base for the Human Glycome Atlas

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The Human Glycome Atlas (HGA) project aims to characterize the human glycome and glycoproteome at cohort scale, generating site-specific glycoproteomic reference maps, glycan structure catalogues, and glycan biosynthesis data. However, glycoscience data commonly exists in separate databases with incompatible formats and identifiers. Researchers must manually link information across these resources, which takes time away from hypothesis generation and discovery. KB-TOHSA is being developed as the knowledge base infrastructure to relieve this burden.A major part of bringing these different data sources together is converting them to the Resource Description Framework (RDF), which represents information through relationships between entities rather than keeping these relationships in separate tables or files. For example, a glycan can be connected to the sites where it occurs, the protein carrying it, the tissue it was observed in, the associated phenotype, and the experiment that produced it. Identifiers will be shared with established resources (GlyTouCan, GlyCosmos, UniProtKB) while community ontologies will be used to represent glycan structures and related concepts. The resulting knowledge graph can be queried directly: a question like "which structures occur at a given site, in which tissues, and in which disease context" can be answered in one integrated query rather than pieced together from separate databases.To ensure the knowledge base is trustworthy and reusable, we are tracking where data came from and how it was processed, validating the structure of the knowledge graph as data is added, and implementing authentication mechanisms to support both open and controlled-access data. Authentication and verifiable credentials are being implemented to support both open data and sensitive data shared selectively with approved collaborators.KB-TOHSA is not a standalone glycan database, but a resource designed to connect HGA data with existing glycoscience databases. It preserves relationships between data points and tracks their origins, enabling researchers to explore connections across datasets, test hypotheses about glycan function in disease, and access structured data for computational analysis.

(P-026) Computational design of de novo Tn antigen-binding proteins using RFdiffusion and molecular dynamics simulations

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Tn antigen (GalNAcα1–O-Ser/Thr) is a tumor-associated carbohydrate antigen that is broadly overexpressed in malignant cells across more than 70% of cancers, while remaining largely absent from the healthy tissue. Designing molecules that specifically recognize the Tn antigen remains difficult due to typically shallow protein-glycan interfaces and water molecules play a key role in glycan binding. In addition to this, the structural diversity and similarity among glycans pose a significant challenge in glycan-binding reagent design, as it requires the development of molecules with high binding specificity to target glycan. To address this challenge, we developed a computational workflow combining deep learning-based protein generation, structural refinement, and molecular dynamics simulation for the de novo design of Tn-binding proteins.Candidate binders were generated using the RFdiffusion (RFD3) framework, with the binding interface directed towards Tn antigen recognition. A large, structurally diverse set of designs was generated to sample a broad range of conformational space. Each candidate underwent structural relaxation in PyRosetta to refine local geometry and eliminate unfavorable steric interactions. Designed proteins were then filtered and ranked using metrics including binding energy, interface stability, shape complementarity, hydrogen bonding patterns, and overall protein stability, allowing identification of high-confidence candidates with favorable energetic and structural properties for molecular recognition. A subset of top-ranked candidates was subsequently subjected to all-atom molecular dynamics simulations in explicit solvent to computationally assess their structural stability and binding affinity towards the Tn antigen. A number of designed proteins show overall stable conformation and strong interaction with the Tn antigen throughout the MD simulations, demonstrating the utility of this generative AI–driven pipeline for glycan-binding protein design.

(P-027) GlyTableMaker: A glycomics and glycoproteomics data repository

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Advances in glycomics and glycoproteomics have enabled detailed characterization of glycan structures, protein glycosylation sites, and glycosylation patterns across organisms, tissues, and cell types. While these technologies generate valuable datasets, the growing scale create significant challenges for data organization, standardization, and reuse. In particular, many of the data generated is published but not deposited into a public data repository making them not easily accessible for the research community or bioinformatics workflows. To address this need, we developed GlyTableMaker, a web-based public data repository designed to streamline the submission of glycomics and glycoproteomics datasets, their standardization and public release. The repository can be used both by data generators as well as curators to deposit their data. GlyTableMaker supports structured entry of glycans, glycoproteins, and site-specific glycosylation data, along with essential biological and experimental metadata, including species, tissue, cell line, disease association, and experimental method used. The platform accommodates both published and unpublished datasets and allows data providers to select data licenses appropriate for their data, allowing for flexibility while encouraging open data sharing. Upon deposition and public release, datasets are freely available in our webportal as well as in machine-readable formats that leverage standardized dictionaries, controlled vocabularies, and ontologies to ensure consistency and interoperability. By simplifying dataset preparation and submission and by standardizing data presentation, the GlyTableMaker repository ensures access to standardized datasets. With a free web portal and machine-readable APIs, GlyTableMaker supports both exploratory and programmatic workflows for data access. GlyTableMaker follows FAIR (Findable, Accessible, Interoperable, and Reusable) data principles and strengthens data sharing and integration within the glycoscience community.

(P-028) Registered but unreachable: what shared glycan identifiers do not resolve

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Glycan researchers routinely begin with a structure and gather what is known about their target glycan: the proteins that carry it, the diseases it accompanies, and other entries in which it has been observed. These resources share GlyTouCan accessions as a common identifier, so this should be automatable. We asked how far it can be.Structures were built and edited with GlycanBuilder-MCP, which allowed us to generate series of glycans differing in exactly one structural element, so that any difference in what could be retrieved was attributable to that element alone. Resources were queried through GlycoNAVI-MCP and TogoMCP, which expose glycan databases and life-science RDF endpoints as callable tools. The resources reached were GlyTouCan, the PDB, GlyCosmos and UniProt. Language models interpreted requests and sequenced operations, but structure construction and identifier resolution remained deterministic. All counts reported here were obtained on 12 August 2026 and are stated as observations of that date.Traversal from a structure to biological context was achievable: a β-GlcNAc structure (G49108TO) reached thousands of glycoproteins in GlyCosmos and, through UniProt, their disease annotations.The limitation lies elsewhere, and is particularly consequential for glycans. Consider 6'-sialyllactose. Among its GlyTouCan entries, two differ only in the configuration at the reducing end: G28868ZC with a β reducing end, and G63717BV with an α reducing end. Neither is more specific than the other; both are legitimate registrations. Searching the PDB returned 12 entries for the first and none for the second. A researcher holding the second accession would conclude that no deposited structure contains it.The same pattern appears with 3'-sialyllactose, where the α and β forms returned 19 and 30 entries; only one entry contains both forms, so 48 distinct entries contain the trisaccharide, but none of the accessions tested retrieves all 48. A distinct case arises when an element is left unspecified. Deposited entries are represented by descriptors in which anomeric configuration and linkage position are specified. An accession with the corresponding element unspecified matches no descriptor directly, and returns nothing.In each case the accession was valid, the query was well formed, yet the result was silently incomplete or empty. Sharing a standard identifier is necessary for cross-resource search but not sufficient. Cross-resource retrieval also requires machine-usable relation coverage and query-time traversal among accessions, distinguishing alternative forms at the same level of specificity from more-specific and less-specific representations. Such traversal would allow a query to be expanded to related entries, rather than only matching against a single descriptor. We outline what this requires, and argue that an answer returned without warning that it is partial is the more dangerous of the two failures.

(P-029) A Tools Suite for Exploring, Navigating and Discovery the Hidden Knowledge of Glyco-Bio KnowledgeBases

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Biomedical knowledgebases such as GlyCosmos, UniProt, ChEBI and our in-development KB-TOHSA of the Human Glycome Atlas project, define biological entities — glycans, glycoproteins, glycogenes, enzymes, pathways, and disease associations — as formal annotated structured data in semantic graphs, where typed relationships between entities are explicitly encoded and machine-interpretable. This formalism enables the ability to enrich data with biological meaning across entities, datasets, and scientific domains and, in principle, the traversal of complex multi-hop biological relationships across heterogeneous datasets within a single integrated framework. In practice, however, this potential is rarely exploited: Query language-based search interfaces present a significant barrier for experimental biologists, while even bioinformaticians familiar with semantic web technologies face graphs of considerable complexity, with meaningful biological connections obscured by ontological depth, dataset fragmentation, and the absence of intuitive navigation tools.We introduce a web-based Tools Suite designed to make the semantic content of glyco-bio knowledgebases discoverable, navigable, and interpretable without requiring prior knowledge of complex schemas and query syntaxes. The suite comprises: an explorer system that looks at a few starting points and maps out everything connected to them in a knowledge graph to surface and highlight connection paths between arbitrary sets of biological entities; an incremental graph browser for step-by-step entity-level exploration; a schema and dataset inspector exposing class hierarchies, property usage, and named graph provenance; a subgraph analytics module; and a guided query construction interface. These components are designed to expose the relational depth of the underlying knowledgebase in biological rather than technical terms, supporting discovery of non-obvious cross-dataset associations — for instance, linking a gene variant through glycoenzyme function, glycan biosynthesis, and glycoprotein modification to a disease phenotype across multiple integrated datasets.Beyond direct research utility, the Suite addresses an emerging need in biomedical AI: large language models and knowledge-grounded AI agents operating over specialized biological domains require access to structured, semantically explicit, contextually anchored relational knowledge. The semantic schemas, annotations, subgraphs surfaced through the tools represent precisely this kind of knowledge — making glyco-bio knowledgebases not only more accessible to human researchers but better positioned as structured knowledge resources for next-generation AI-assisted biological discovery.The Tools Suite is developed against GlyCosmos (glycosmos.org) as primary testbed

and is designed for deployment within the KB-TOHSA Portal of the Human Glycome Atlas project, where it serves as a core component alongside the other Portal discovery interfaces and tools.

TOPIC 3: Biomarkers, Diagnostics, and Precision Glycomedicine

(P-030) EP- and USP-Compliant Separation of Fluorodeoxyglucose from Fluorodeoxymannose and Chlorodeoxyglucose by Anion Exchange Chromatography with Pulsed Amperometric Detection

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Fluorodeoxyglucose (FDG) is a glucose analogue that, when labelled with fluorine-18, becomes a radioactive tracer, 18F-FDG. 18F-FDG is used in positron emission tomography (PET) to identify tissues with altered glucose metabolism. Altered glucose metabolism is associated with malignancies, epilepsy, Alzheimer's disease and more1. Substitution of the hydroxyl group at the C2 position of FDG with 18F results in 18F-FDG, once transported into the cell it is into FDG-6P, which cannot be further phosphorylated or dephosphorylated. Therefore, 18F-FDG accumulates in these diseased tissues, making them detectable during PET imaging by annihilation photons during the subsequent decay of 18F. Due to 18F-FDG short half-life of 109.8 minutes, 18F-FDG is produced in a cyclotron near the PET facility. The synthesis begins with a protected mannose precursor, which undergoes a nucleophilic substitution with 18F, resulting in inversion at the C2 carbon and conversion of the mannose derivative into FDG. A subsequent hydrolysis step removes the protecting groups to produce the final 18F-FDG product 2. The synthesis typically yields approximately 95% 18F-FDG; however, small amounts of impurities can also be produced depending on the synthesis route. One common impurity is 2-fluoro-2-deoxy-D-mannose (FDM), an epimer of FDG that may form as a by-product during fluorination. Another is 2-deoxy-2-chloro-D-glucose (CDG), which can remain if the chloride atom on the precursor is not completely replaced by fluoride during the nucleophilic substitution step. CDG may also be generated during the hydrolysis stage when hydrochloric acid is used to remove the acetyl protecting groups.The quality requirements for 18F-FDG are outlined in both the United States Pharmacopeia (USP) and the European Pharmacopoeia (EP), which require chemical purity testing before the radiopharmaceutical can be administered to patients. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is the preferred analytical technique for detecting these impurities due to its high sensitivity and ability to distinguish between structurally similar carbohydrate compounds. We present data that shows excellent separation of FDG from its impurities, CDG and FDM, using a Thermo Fisher Scientific TM Dionex TM CarboPac TM PA20 fast 4 µm column on a ICS-6000 system according to the EP and USP criteria. The method allows for fast and reliable routine separation with excellent resolution.

(P-031) Protective role of immunoglobulin N-glycan against disseminated intravascular coagulation in urosepsis

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Introduction and Objectives: Disseminated intravascular coagulation (DIC) is a severe and often fatal complication of sepsis, characterized by systemic activation of coagulation pathways and widespread microvascular thrombosis. In the context of urosepsis, early identification of patients at risk for DIC is critical for timely intervention and improved outcomes. Immunoglobulin N-glycans, which are post-translational modifications of antibody molecules, play diverse roles in immune regulation, inflammation, and endothelial integrity. Despite their biological significance, the relationship between serum immunoglobulin N-glycosylation patterns and the development of DIC in sepsis remains poorly understood. This study aimed to investigate whether specific N-glycan profiles are associated with DIC onset in patients with urosepsis, and to explore their potential mechanistic link to endothelial dysfunction.Materials and Methods: This retrospective study included 145 patients diagnosed with urosepsis. DIC was defined according to the criteria of the Japanese Association for Acute Medicine. Serum concentrations of 26 distinct immunoglobulin N-glycans were quantified using automated capillary electrophoresis-based N-glycomics. Multivariable logistic regression analyses

were conducted to assess the association between N-glycosylation profiles and DIC development.Results: The median patient age was 79 years. Among the 145 patients, 26 (18%) developed DIC. Of the 26 N-glycans analyzed, two species—G0N[6] and G0F-N[6]—showed significant differences between patients with and without DIC (Fig. A and C). Patients with higher levels of these glycans had significantly lower rates of DIC compared to those with lower levels (Fig. B and D). The structural characteristics of these glycans are illustrated in Figure E. After adjusting for qSOFA scores, elevated levels of the asialo, agalacto truncated biantennary glycan with core fucose (G0F-N[6]) remained significantly associated with reduced DIC risk (Table). Furthermore, G0F-N[6] levels were significantly correlated with serum E-selectin concentrations, a marker of endothelial dysfunction (Fig. F).Conclusions: The asialo, agalacto truncated biantennary glycan with core fucose (G0F-N[6]) in serum immunoglobulin may exert a protective effect against DIC development in urosepsis, potentially through the preservation of endothelial function.

(P-032) Differential effects of testosterone deprivation and androgen receptor inhibition on endothelial glycocalyx integrity in prostate cancer

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Background:Endothelial glycocalyx, which coats the luminal surface of vascular endothelial cells, plays a pivotal role in maintaining vascular homeostasis by regulating thrombogenicity, modulating inflammatory responses, and preserving endothelial barrier integrity. Androgen deprivation therapy (ADT) and androgen receptor signaling inhibitors (ARSIs) are associated with cardiovascular risk in prostate cancer. However, their effects on endothelial glycocalyx integrity, an early determinant of endothelial dysfunction, remain unknown. We investigated whether ARSIs or ADT induce endothelial glycocalyx injury.Methods:In this retrospective study, 43 patients initiating ARSIs (enzalutamide, abiraterone acetate, apalutamide, or darolutamide) and 39 patients initiating ADT (LHRH agonists, GnRH antagonists, or combined androgen blockade) were analyzed. Plasma hyaluronan levels, a validated biomarker of endothelial glycocalyx injury, were measured before and 1 month after treatment initiation using enzyme-linked immunosorbent assay. Paired comparisons were performed using the Wilcoxon signed-rank test.Results:Plasma hyaluronan levels did not significantly change after ARSI initiation (mean 260 vs 270 ng/mL, P = 0.724). In contrast, ADT initiation was associated with a significant increase in hyaluronan levels (mean 238 vs 420 ng/mL, P = 0.009). No significant differences in plasma hyaluronan changes were observed between LHRH agonists and GnRH antagonists (P = 0.816). Conclusions:Short-term ARSI therapy did not impair endothelial glycocalyx integrity, whereas ADT was associated with significant glycocalyx injury. These findings suggest that testosterone deprivation, rather than direct androgen receptor inhibition, may contribute to endothelial dysfunction during prostate cancer treatment.

(P-033) Rapid and reversible endothelial glycocalyx disruption during tyrosine kinase inhibitor therapy in locally advanced or metastatic renal cell carcinoma

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Background:Endothelial glycocalyx, which coats the luminal surface of vascular endothelial cells, plays a pivotal role in maintaining vascular homeostasis by regulating thrombogenicity, modulating inflammatory responses, and preserving endothelial barrier integrity. Tyrosine kinase inhibitors (TKIs) are associated with vascular toxicities, yet their impact on endothelial glycocalyx integrity remains unclear. Because glycocalyx disruption is an early event in endothelial dysfunction, we investigated whether TKI therapy induces endothelial glycocalyx injury in patients with locally advanced or metastatic renal cell carcinoma (la/mRCC).Methods:In this retrospective study, 25 patients initiating and 24 patients discontinuing TKIs (axitinib, cabozantinib, lenvatinib, or pazopanib) due to adverse events were analyzed. Plasma hyaluronan levels, a validated biomarker of endothelial glycocalyx injury, were measured before and 1 month after TKI initiation or discontinuation using enzyme-linked immunosorbent assay. Paired comparisons were performed using the Wilcoxon signed-rank test. Differences among agents were evaluated using the Kruskal–Wallis test.Results:Among patients initiating TKIs, plasma hyaluronan levels significantly increased at 1 month (median 117 vs. 218 ng/mL; +75%; P<0.001). No significant differences were observed among the four agents (P = 0.922). Conversely, in patients discontinuing TKIs due to adverse events, plasma hyaluronan levels significantly decreased (median 221 vs. 135 ng/mL; −37%; P<0.001), without inter-agent differences (P = 0.865).Conclusions:TKI therapy is associated with rapid and reversible

endothelial glycocalyx injury in patients with locally advanced or mRCC. These findings suggest a potential mechanistic link between TKI exposure and vascular toxicity across agents.

(P-034) Detection of Anti-Glycan Antibodies in Saliva and Serum Using GlycoStrips

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Detection of anti-glycan antibodies normally requires sophisticated instrumentation and specialized laboratory environments along with technical expertise in running such devices. However, characterizing anti-glycan antibodies can play an important role in transfusion medicine, transplantation and infectious diseases. Such characterization would benefit from rapid and easy detection methods which can be adopted even in remote areas with little-to-no expertise. For this reason, we sought to develop a versatile, instrument-free, membrane-based microarray platform called GlycoStrip that is capable of rapid, visual antibody profiling at the point of care using alpha-numeric patterns which can detect the presence of anti-glycan antibodies within 30 minutes. To demonstrate the utility of the platform, we printed neoglycoproteins with blood group antigens (ABO) as well as pathogen-associated proteins (COVID Spike Proteins) and whole bacteria. We tested these strips with purified proteins (lectins and antibodies), human saliva for IgA detection and human serum for IgG. The platform successfully resolved distinct anti-glycan IgA and IgG repertoires, allowing for the rapid inference of ABO blood group status from saliva and serum, respectively. Furthermore, the platform demonstrated high sensitivity in detecting IgG-mediated responses to intact bacterial cells and pathogen-associated proteins such as the COVID spike proteins. The antibody binding profiles generated by this paper-based system showed high correlation with established high-resolution microarray and Luminex standards, demonstrating that high-density epitope presentation can be effectively translated to a simplified, portable format. GlycoStrip is a transformative technology for the rapid and visual detection of a wide range of immunogenic targets, and is easily expandable using additional glycan and non-glycan targets. This work decouples antibody profiling from complex laboratory infrastructure, providing a scalable solution for real-time immunological monitoring, diagnostic screening, and personalized medicine in both clinical and resource-limited settings.Reference: Jia T, Mehta AY, Charon KM, Noel M, Singh P, von Gunten S, Roback JD, Heimburg-Molinaro J, Stowell SR, Cummings RD. Point-of-care GlycoStrips detect serum and salivary antibodies to blood group antigens and pathogens. Trends Biotechnol. 2026.DOI: 10.1016/j.tibtech.2026.06.019. PMID: 42457473; PMCID: PMC13378435.

(P-035) Serum N-glycan profiling as a potential biomarker for biopsy-proven rejection after living-donor kidney transplantation

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Background: We investigated whether serum N-glycan profiles could serve as biomarkers for early risk stratification of graft rejection after living-donor kidney transplantation (KT).Methods: We retrospectively analyzed 174 living-donor KT recipients from five medical centers. Serum N-glycan levels were measured in postoperative samples using glycoblotting coupled with mass spectrometry. Integrated prediction scores for graft rejection were developed using recipient age, sex, immunological risk factors, and serum N-glycan levels measured on post-transplant days (D) 1 and 7. The association between the combined D1 and D7 score and rejection-free survival was assessed using Kaplan–Meier analysis.Results: Among the 174 recipients, 52 experienced graft rejection, whereas 122 did not. Integrated scores at both D1 and D7 were significantly higher in recipients who subsequently

developed rejection than in those who remained rejection-free. The areas under the receiver operating characteristic curve (AUCs) for the integrated scores at D1 and D7 were both 0.84. A combined D1 + D7 score of ≥0.50 identified recipients who developed graft rejection with 81% sensitivity and 80% specificity, with an AUC of 0.87. Recipients with a combined score ≥0.50 had significantly shorter rejection-free survival than those with lower scores.Conclusions: Serum N-glycan profiling, when combined with clinical and immunological factors, may facilitate early identification of kidney transplant recipients at increased risk of graft rejection.

(P-036) Matrix Metalloproteinase Signatures and their Association with Glycocalyx Injury and Fluid Overload After Pediatric Cardiac Surgery with Cardiopulmonary Bypass

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Background: Endothelial glycocalyx injury, as measured by plasma syndecan-1 concentrations, has been associated with vascular hyperpermeability and fluid overload following cardiopulmonary bypass (CPB). While regulated by tissue inhibitor of metalloproteinases (TIMPs), matrix metalloproteinases (MMPs) are known to degrade the glycocalyx and excessive release of specific MMPs in response to CPB could represent therapeutic targets to prevent bypass induced glycocalyx injury. We aimed to identify unique MMP signatures in children after exposure to CPB and their association to markers of endothelial glycocalyx injury, postoperative fluid overload and related ICU outcomes. Methods: We included 26 children from two major children's hospitals undergoing cardiac surgery with CPB. Plasma was collected pre- and postoperatively on admission to the cardiac ICU. Plasma concentrations of MMPs 1, 3, 7, 8, 9, 12, 13, TIMP1 and syndecan-1, -4 were quantified using a multiplex assay. Additionally, a fluorimetric MMP activity assay was performed on a subset of our cohort to quantify general MMP enzymatic activity. Plasma concentrations and MMP/TIMP1 ratios were compared to cumulative peak percent fluid overload, markers of glycocalyx injury and ICU related outcomes. Results: A total of 26 children were included in the primary analysis. Median postoperative peak percent fluid balance was 11.1 (IQR 6.7- 16.2) among the cohort. Syndecan-1 and -4 concentrations were associated with peak percent fluid balance, p=0.016 and p=0.033 respectively. Postoperative MMP 7, 9, 13 and TIMP1 concentrations were significantly elevated after CPB exposure, p<0.001, p=0.005, p=0.002 and p<0.001 respectively. MMP 7 (rs 0.40, p=0.042) and MMP 13 (rs 0.44, p=0.023) concentrations were positively correlated with time on bypass. Fluorimetric MMP activity assays revealed general MMP activity was overall decreased postoperatively, however specific MMP/TIMP1 concentration ratios were significantly correlated to syndecan shedding, peak percent fluid overload, vasoactive infusion score, duration of postoperative mechanical ventilation and ICU length of stay with variation by MMP type. Conclusions: Specific MMP signatures in response to CPB exposure may be associated with postoperative fluid overload and its associated poor outcomes in children undergoing cardiac surgery. Further studies exploring the mechanistic impacts of MMPs on physiologic fluid shifts, clinically significant fluid overload and postoperative outcomes is needed.

(P-037) Frozen Oral Tissue Glycoproteomics for Cancer Biomarker Discovery

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Oral squamous cell carcinoma (OSCC) is a highly aggressive subtype of head and neck squamous cell carcinoma, accounting for approximately 90% of oral cancer cases. Globally, approximately 422,000 new cases and more than 208,000 deaths occur annually from OSCC. Further, the incidence of OSCC is projected to increase by 61% by 2040, accompanied by a continued rise in mortality worldwide. Despite advances in treatment, OSCC remains associated with poor clinical outcomes, with a 5-year survival rate of

approximately 55%, highlighting the urgent need for biomarkers for oral cancer early detection. Previous research has demonstrated alterations in oral glycoproteins during epithelial-to-mesenchymal transitions (EMT), suggesting that these changes may serve as accessible signatures for early-stage screening. The purpose of this study is to develop a method to survey and identify potential glycoprotein biomarker candidates across non-cancer tissue, premalignant lesions, and OSCC. Human tissue, including 10 non-cancerous, 10 premalignant, and 9 OSCC samples, were homogenized using a BeatBox, then underwent proteolysis with trypsin protease, followed by peptide cleanup (PreOmics). For each lysate digest, 90% was subjected to glycopeptide enrichment (AffiniSep), while the remaining 10% was reserved for proteomic analyses. The resulting peptides were subsequently analyzed by nano-liquid chromatography tandem mass spectrometry (nLC-MS/MS) using HCD for proteomic analyses and HCD product-dependent EThcD (HCD-pd-EThcD) for glycopeptide analyses. Peptides and proteins were assigned using the SEQUEST algorithm and FragPipe software. In OSCC, oxidative phosphorylation (adj. p=3.2E-10) and fatty acid metabolism (adj. p=7.4E-8) pathways were enriched in comparison to non-cancer based on pathway enrichment analyses of the 'hallmark' gene set from the Molecular Signatures Database. These trends are consistent with previously established metabolic changes in OSCC. Across individual data sets, over 2500 unique proteins (≥2 peptides, 1% FDR), over 330 N-glycopeptides, and greater than 140 O-glycopeptides were assigned. Combined analysis of non-cancer, pre-cancer, and OSCC resulted in over 1000 N-glycopeptides with 129 unique glycan compositions and representing 206 unique glycoproteins. Together, these findings demonstrate that the workflow provides deep complementary characterization across protein, N-glycopeptide, and O-glycopeptide space. This supports the utility of this approach for systematic glycoproteomic investigation and biomarker discovery. In the future, quantitative analyses will be performed by comparing glycopeptide areas and relative abundances across non-cancerous, premalignant, and OSCC tissue samples.

(P-038) Integration of N-glycan mass spectrometry imaging and spatial proteomics for the analysis of pancreatic neoplasms and mucinous tumor tissues

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Intraductal papillary mucinous neoplasms (IPMNs) are common mucin-producing precursor lesions of pancreatic ductal adenocarcinoma (PDAC) that progress through stages of dysplasia and can ultimately develop into invasive carcinoma. Because current clinical and pathological features of IPMN lesions have limited ability to predict individual lesion behavior, there is a critical need for molecular biomarkers that can improve risk stratification and early detection. In this study, FFPE human IPMN tissues (n = 21), representing all grades of dysplasia, were analyzed using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to characterize lesion-associated N-glycans and compare them with adjacent mucinous pancreatic cancers.More than 200 N-glycans were analyzed across the cohort. While the majority of N-glycans identified in IPMN tissues were also present in PDAC lesions, differences in glycan abundance were observed. IPMNs were enriched in lower-mass N-glycans (1500-1800 m/z) containing bisecting GlcNac, whereas cancer lesions exhibited greater abundance of larger bisecting, branched and fucosylated bisecting glycans.IPMN tissues also revealed an enrichment of LacdiNAc (GalNAc-GlcNAc) motifs. Certain IPMN and mucinous tumor tissues contained notably higher numbers of LacdiNAc-bearing N-glycans, with up to 50 distinct species identified within a single tissue section. These glycans displayed unique spatial distributions and were evaluated relative to their more common lactosamine (Gal-GlcNAc) structural counterparts. Pancreatic islets, when present, exhibited distinct N-glycan profiles characterized by sulfated bi-antennary LacdiNAc structures.A subset of 12 tissues underwent cyclic IF staining of four cellular markers followed by GeoMx spatial proteomic analysis of 77 proteins in pathologist-selected ROIs representing low-grade IPMN, high-grade IPMN, and PDAC. Using QuPath and SCiLS Lab software, each ROI was co-registered onto the N-glycan MALDI-MSI slides to extract glycan peak intensity data for heatmap comparisons. PLSDA demonstrated clear disease clustering and identified the glycan species contributing most strongly to pathological classification. These glycan signatures were then compared with spatial protein expression profiles from the same ROIs to assess associations between glycan abundance, protein expression, and histopathological features.Overlap between IPMN and PDAC glycomes suggests that glycomic remodeling accompanies progression toward invasive disease. These results establish spatial glycomics as a promising approach for identifying biomarkers of malignant potential and lay the foundation for future integration of other spatial omic analyses. Ongoing studies are investigating associations between LacdiNAc expression, histopathologic subtype, grade of dysplasia, and clinical outcomes to assess its utility as a biomarker of progression.

TOPIC 4: Cancer Glycobiology and Therapeutics

(KEY4-001) Targeting glycosylation for precision oncology

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Altered glycosylation in tumours and their microenvironment promotes disease progression, in part through cancer-associated glycans and lectin receptors that function as glyco-immune checkpoints regulating immune recognition and tolerance [1–3]. Truncated O-GalNAc glycans, including Tn and sialyl-Tn (STn), are broadly expressed in carcinomas but largely absent from healthy epithelia, creating cancer-selective therapeutic targets [2,3]. This presentation will report two complementary approaches to aberrantly O-glycosylated surface antigens in solid tumours. First, AM52.1 is a novel STn-specific mAb. AM52.1 CAR-T cells showed target-specific cytotoxicity, tumour regression and prolonged survival in gastric, tubo-ovarian and colorectal cancer mucinous peritoneal metastasis models. It also targeted STn-positive patient-derived cancer organoids while sparing normal-tissue organoids [4]. Second, a glycopeptide-epitope strategy generated several cancer-specific antibodies to protein-defined Tn glycoforms. One example is the Tn-CD44v6-specific mAb 4C8, which was used to generate CAR-T cells. The 4C8 CAR-T cells showed target-specific cytotoxicity, tumour regression and prolonged survival in PDX models. Moreover, 4C8 CAR-T cells, selectively killed targets in a mixed organotypic skin-cancer model while sparing healthy keratinocytes [5]. In addition, several humanized glyco-peptide specific mAbs have been created targeting the Tn version of MUC1 (GO-M100B) and MUC4 (GO-M400). Both mAbs were developed as antibody-drug conjugates (ADCs), and showed low-nanomolar binding, rapid internalization and selective recognition of Tn-positive tumour cells [6]. IHC showed broad and selective epithelial-cancer detection, and neither ADC was cytotoxic to normal primary cells. The Tn-MUC1 ADC (GO-M100B-vedotin) was active across 22 PDX/CDX models. In nine models M100B-vedotin demonstrated more than 90% tumour growth inhibition, including 3 models showing complete regression. Similar efficacy was observed with GO-M400-vedotin in tested models. Both ADCs demonstrated minimum effective doses of 1.5-3 mg/kg. Furthermore, when formulated with the topoisomerase inhibitor, exatecan, GO-M100B outperformed the recently developed ADC targeting MUC1 (Ds-3939) from Daiichi Sankyo. Pilot non-human-primate studies showed tolerability and favourable pharmacokinetics [6]. Patient-derived organoids and organotypic human-tissue models provided complementary readouts of tumour selectivity. Together, these studies establish tumour-specific glycoforms as actionable targets for CAR-T-cell and ADC therapies.[1] Mereiter S, et al. Cancer Cell. 2019 Jul 8;36(1):6-16. [2] Aasted, Mikkel; et al. Chemical Reviews 2026. doi:10.1021/acs.chemrev.5c00901.[3] Pires J, et al. Glycobiology. 2026 Jul 31;36(9):cwag049. [4] Abrantes R et al. Cell Rep Med. 2025;6:102350.[5] Aasted MKM et al. Mol Cancer Ther. 2023;22:1204-1214.[6] Shrestha N et al. AACR 2026; Poster 6923.

(PT-004) Deciphering Siglec ligands in cancer to improve immunotherapy

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Aberrant sialylation is a hallmark of cancer, contributing to immune evasion, metastasis, and drug resistance. Siglecs are a family of fifteen carbohydrate-binding proteins broadly expressed on the surface of immune cells and have recently emerged as glyco-immune checkpoints, as their sialylated ligands are upregulated on cancer cell surfaces. The Siglec-sialic acid axis is a promising target for immunotherapy, and efforts are underway to develop therapies that block Siglec-sialic acid interactions. However, major challenges remain in precisely determining the specific carbohydrate ligands of Siglecs. These ligands are difficult to study using conventional tools because: (i) these interactions are biochemically weak, (ii) carbohydrate structures cannot be directly predicted from the genetic code and (iii) they are very chemically-complex and it may not simply be a single structure that is a ligand. Advanced tools enhancing the avidity and stability of these interactions are needed to map Siglec-glycan landscapes on cancer cells. Recently, the Macauley lab improved methods to detect Siglec ligands by designing sensitive and quantitative methods and the use of recombinant soluble Siglecs. Using our cutting-edge biochemical tools, we have profiled Siglec ligands on the entire NCI-60 panel of cancer cells. We

identified distinct Siglec binding patterns, providing insights into how cancer-specific sialylation signatures may influence Siglec interactions. We have compared this dataset to transcriptomics and used our comprehensive knowledge of the ligands for Siglecs to simulate 'turn-on' and 'turn-off' pathways for Siglec ligand biosynthesis. For four Siglecs, we now can accurately predict if cancer cells have ligands for each Siglec member, using a multi-factorial prediction algorithm based on transcriptomics data. Ongoing overexpression and CRISPR-Cas9 knockout experiments are being conducted to validate these predictions by assessing the impact of specific glyco-genes on Siglec ligand expression in cancer cells. Simultaneously, we are producing recombinant sialyltransferases to synthesize and study sialylated glycoconjugates in a controlled environment. This facilitates the biosynthesis of Siglec ligands on cell surface to validate sialyltransferases role in Siglec-sialic acid axis. Based on the generated predictive algorithms, we are conducting validation on clinical samples, particularly for patients not responding to current immunotherapies, which we hypothesize have higher levels of Siglec ligands. This approach underscores the potential of targeting the Siglec-sialic acid axis to identify novel cancer biomarkers and improve personalized immunotherapy.

(PT-005) Dietary Fructose Directly Fuels N-Linked Glycosylation to Promote Colorectal Cancer Progression

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Aberrant N-linked glycosylation is a hallmark of cancer and can alter protein folding, trafficking, receptor signaling, cell adhesion, and tumor–immune interactions, yet how dietary nutrients directly contribute to glycan biosynthesis in cancer remains poorly understood. Dietary fructose has been linked to colorectal cancer (CRC) progression, and previous work showed that high-fructose corn syrup (HFCS) accelerates intestinal tumor growth through ketohexokinase (KHK)-dependent metabolism. However, little fructose-derived carbon enters glycolysis in tumor cells when glucose is abundant, suggesting that fructose may support tumorigenesis through alternative metabolic fates. We therefore investigated whether dietary fructose directly fuels N-linked glycosylation in CRC. Spatial N-glycomic imaging by MALDI mass spectrometry revealed that HFCS exposure broadly remodels intestinal and tumor N-glycosylation in wild-type and APC-mutant mice, with increased high-mannose and core α1,6-fucosylated glycans. Stable isotope tracing with [U-13C]-fructose demonstrated direct incorporation of fructose-derived carbon into high-mannose N-glycans in vivo, while GLUT5-deficient mice showed markedly reduced labeling, supporting a direct contribution of intestinal fructose uptake to glycan biosynthesis. Mechanistically, we identified mannose phosphate isomerase (MPI), a central enzyme in mannose and GDP-mannose metabolism, as a previously unrecognized fructose-1-phosphate (F1P)-binding protein. Molecular docking and biochemical analyses showed that MPI binds both F1P and fructose-6-phosphate, while enzymatic assays demonstrated that MPI can convert F1P to mannose-1-phosphate, revealing a previously undescribed route linking fructose metabolism to glycan precursor synthesis. Consistent with this pathway, fructose increased high-mannose N-glycosylation in HCT116 and DLD1 cells, two CRC cells lines, under glucose restriction and hypoxia. Increasing F1P catabolism through aldolase B overexpression abolished fructose-mediated enhancement of hypoxic survival, whereas CRISPR-mediated MPI loss slowed proliferation, produced a mannose-dependent growth defect, and impaired fructose-mediated rescue of hypoxic survival. MPI loss also decreased phospho-STAT3 signaling, while tumors from HFCS-exposed APC-mutant mice showed increased phospho-STAT3, suggesting that fructose-driven glycan remodeling may alter signaling pathways relevant to tumor progression. Together, these findings identify N-linked glycosylation as a major metabolic destination of dietary fructose and establish a direct biochemical link between fructose metabolism and glycan biosynthesis. They further identify MPI-dependent fructose metabolism as a potential therapeutic vulnerability in fructose-driven CRC.

(PT-006) KLK5-secreting CAR-T cells remodel the tumor glycocalyx to enhance antitumor activity in solid tumors

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CAR-T cell therapy has transformed treatment of hematologic malignancies, yet its efficacy in solid tumors remains limited by physical and biological barriers within the tumor microenvironment. Although most CAR engineering strategies focus on antigen recognition and intracellular signaling, effective cytotoxicity also requires close membrane apposition between CAR-T cells and tumor cells. We previously identified the cancer cell glycocalyx, a dense layer of cell-surface glycans and mucins, as a nanoscale barrier that restricts immune-cell engagement. Using scanning angle interference microscopy (SAIM), we showed that modest changes in glycocalyx thickness alter susceptibility to NK- and CAR-T cell-mediated killing. These findings motivated the development of glycocalyx-editing CAR-T cells that locally remodel the mucin barrier in solid tumors. Using a previously established doxycycline-inducible MCF10A Tn-MUC1 model, we found that increasing Tn-MUC1 expression increased antigen abundance but reduced CAR-T killing, demonstrating that dense mucins can act as both target antigens and physical barriers. Extending this concept to our MUC17 CAR-T model, StcE mucinase treatment increased CAR-T avidity toward MUC17-expressing tumor cells and improved tumor-cell killing. Together, these results established enzymatic glycocalyx remodeling as a strategy to enhance CAR-T engagement. Because the bacterial origin of StcE may limit clinical translation, we screened human kallikrein-family proteases and identified KLK5 as a glycocalyx-editing candidate. Although KLK5 is a serine protease rather than a canonical mucinase, it cleaved multiple glycocalyx components, including MUC1, MUC16, MUC17, and CD43. Sialidase pretreatment abolished this activity, indicating that KLK5-mediated mucin cleavage is sialylation-dependent under these conditions. KLK5 also reduced cell-surface FGF2 binding, consistent with decreased functional availability of heparan sulfate and broader glycocalyx remodeling. In an exploratory intravenous dose-escalation study, no treatment-associated tissue lesions were detected under the tested conditions. We then engineered MUC17-, mesothelin-, and Tn-MUC1-targeted CAR-T cells to secrete KLK5 locally at the tumor interface. These cells were evaluated in gastric and pancreatic cancer models using GSU, ASPC-1, and Capan-2 cells. KLK5-secreting CAR-T cells improved antitumor activity in vitro and enhanced tumor control relative to conventional CAR-T cells in GSU and ASPC-1 xenograft models. Together, these findings establish the mucin-rich glycocalyx as an actionable barrier to CAR-T cell therapy. Engineering CAR-T cells to secrete a human glycocalyx-editing protease improved tumor-cell engagement without altering CAR specificity and enhanced activity across multiple mucin-rich tumor models. This strategy may provide a broadly applicable approach for overcoming glycocalyx-mediated resistance in solid tumors.

(P-039) Novel artificial lectin derived from gut bacteria fused to Fc as a therapeutic for highly mannosylated targets

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Aberrant glycosylation is a feature of many cancers, which present unique glycan signatures relative to normal cells. One such aberration is high-mannose linkages consisting of five to nine mannose residues1, which can potentiate increased migration and invasion in cancer cells . In healthy cells this structure is truncated and transformed to complex branched type glycans in the endoplasmic reticulum by mannosidases. We have previously described an endoglycosidase, BT1285, derived from B.thetaiotaomicron, that cleaves high-mannose (HM) N-glycans2. By introducing inactivating mutations in the active site of BT1285, we have produced an artificial lectin BT1285i that does not have enzymatic activity but retains high affinity binding to oligomannose substrates. We generated a construct of BT1285i fused to the Fc fragment of IgG1 to create a biologically active therapeutic that can elicit cytotoxic effector function, interact with immune cells and maintain long half-lives through interactions with its cognate Fc receptors3. We produced the BT1285i-Fc fusion protein in a mammalian system to ensure glycosylation of the Fc fragment, thereby preserving its effector functions4. Here we report the design, production and validation of a novel artificial lectin fused to Fc that binds with high specificity and affinity to cancer cells that were previously described as being enriched in high oligomannose glycoproteins at the cell surface as well as to an HM-overexpressing variant of HEK293F cells. Our biophysical assays demonstrate that BT1285i-Fc is thermally stable. By flow cytometry, we show that BT1285i-Fc binds with nanomolar affinity to HM-HEK293F, as well as with micromolar affinity to the NSCLC cell line. This effect is abrogated by treatment with an endoglycosidase specific to HM N-glycans, demonstrating that the binding is specific to the presence of HM glycans on these cells. These findings suggest that these highly specific artificial lectins can be exploited to therapeutically target oligomannosylated cancer cells.

(P-040) RUNX1 orchestrates glycocalyx structure in Colo205 colorectal cancer cells

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RUNX1 is a transcription factor with recognized importance in hematopoiesis and leukemia development. However, studies from independent groups have shown that RUNX1 also regulates colonocyte differentiation, and its expression may be related to a poor prognosis in some epithelial cancers, including subtypes of colorectal cancer (CRC). Previously, we identified RUNX1 as a regulator of cholera toxin ligand expression in Colo205 cells (a CRC cell line), but so far, no studies have explored RUNX1 targets that shape cell surface glycosylation. Thus, considering the importance of the glycocalyx for cancer and biological processes such as differentiation and adhesion, we investigated RUNX1-dependent changes in cell surface glycosylation in Colo205 cells and explored cancer-relevant outcomes directed by this transcription factor. We found that RUNX1 knockout (KO) Colo205 cells present significant changes in cell surface glycosylation, including increased expression of the epitope Lewis X (LeX) and decreased expression of its sialylated form, sialyl Lewis X (sLeX). Reduced levels of both ST3GAL4 and CD43 transcripts were detected in the KO cell line. However, sLeX expression could be restored in RUNX1 KO cell line through expression of ST3GAL4, indicating that expression of the sialyltransferase and not the glycoprotein scaffold, may be responsible for RUNX1-dependent sLeX expression. RUNX1 KO cells exhibited decreased binding to SIGLEC9, suggesting that RUNX1 expression might inhibit phagocytosis but in vitro assays did not confirm this hypothesis. RUNX1 KO cells also showed reduced binding to E-selectin, implying that RUNX1-dependent glycosylation could contribute to the cell-cell interaction capacity of Colo205 cells. Indeed, homotypic cell-cell aggregation was dramatically increased in RUNX1 KO cells. However, expression of ST3GAL4 and CD43 did not reverse cell-cell aggregation, suggesting that the aggregation phenotype was not due to the change in sialylation. Increased expression of multiple cadherins and protocadherins was observed in RUNX1 KO cells, Further, the cell-cell aggregation observed in RUNX1 KO cells could be blocked using an antibody against E-cadherin although this specific cadherin was not upregulated in the RUNX1 KO cells. Current efforts focus on studying the roles of cadherin and protocadherins, as well as their post-translational modifications, in RUNX1-regulated changes in cell-cell adhesion. Taken together, our results demonstrate that RUNX1 functions as a master regulator of glycosylation in colorectal cancer cells and thereby controls cancer-related phenotypes including cell-cell adhesion. Support: Mary Kay Charitable Foundation, NIH R35GM145599.

(P-041) Role of ST6GAL1 Sialyltransferase in Pancreatic Cancer Immune Evasion

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy. A key factor contributing to the lethality of PDAC is immune evasion. Tumor cells suppress anti-tumor immunity by activating immune checkpoint proteins on immune cells including macrophages. Siglecs are one of the main checkpoint molecules on macrophages. The ligand for Siglecs is sialic acid. To effectuate immune suppression via Siglecs, PDAC cells increase their surface sialylation by upregulating the expression of sialyltransferases such as ST6GAL1. ST6GAL1 adds an α2,6-linked sialic acid to N-glycans. Both α2,6 sialylation and ST6GAL1 expression are markedly increased in PDAC cells. Our prior studies revealed potent tumor-autonomous functions for ST6GAL1. Our unpublished results show that the tumorigenic effects of ST6GAL1 are also driven by its role in generating sialoglycan ligands for macrophage Siglecs. Macrophages that are polarized to an immunosuppressive M2 phenotype are key contributors to PDAC progression. The goal of our study is to determine whether macrophage Siglecs are promising therapeutic targets for preventing M2 polarization, thus restoring anti-tumor immunity and preventing PDAC progression. We investigated macrophage polarization in our genetically engineered mouse models of PDAC, utilizing either pancreas-specific knock-in of oncogenic K-ras (KC mouse) or K-ras in combination with ST6GAL1 knock-in (KSC mouse). Single-cell RNA sequencing of KSC and KC pancreata identified a distinct M2-like tissue-associated macrophage (TAM) cluster expressing Trem2, Spp1, Apoe, and Arg1 genes. Compared with KC mice, KSC mice showed enrichment of this M2-like TAM population, with IL-10 signaling identified as the top canonical pathway by IPA and epithelial-mesenchymal transition enriched by GSEA. DEG analysis of KSC vs KC pancreata in that TAM cluster further confirmed increased expression of M2-like genes. These findings were supported by flow cytometry and immunohistochemistry, demonstrating increased M2-like macrophages in KSC pancreata. Next, macrophages that co-cultured with ST6GAL1-

overexpressing PDAC cells showed increased expression of M2 markers. But adding Siglec-blocking antibodies reversed the ST6GAL1-mediated M2 polarization. Phagocytic capability of macrophages was suppressed by ST6GAL1-overexpressing PDAC cells and recovered by a Siglec-blocking antibody. Cytokine array analysis showed that macrophages co-cultured with ST6GAL1-overexpressing PDAC cells exhibited increased expression of M2 macrophage-associated cytokines. Overall, our data demonstrate that α2,6 sialic acids on PDAC cells engage with Siglecs on macrophages to induce polarization of macrophages into an immunosuppressive M2 phenotype. Collectively, these results reveal a potential mechanism for targeting Siglecs as a promising immune checkpoint therapy for PDAC patients with high levels of ST6GAL1.

(P-042) Overexpression of ST6GAL1 in Murine CRC leads to sialylation of GLUT1 and alters Metabolism

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Colon and rectal cancer (CRC) is the third most frequently diagnosed cancer in the United States and remains a major cause of cancer-related mortality. Increasing evidence suggests that aberrant glycosylation plays a role in tumor progression and immune modulation in pancreatic, ovarian and CRC. ST6GAL1, a sialyltransferase, catalyzes the addition of sialic acid to N-glycosylated proteins, altering their function. We hypothesized that overexpression of ST6GAL1 in the murine CRC cell line, CT26, could alter cellular metabolism via sialylation of GLUT1. To begin, our murine CRC CT26 cells were transduced with an ST6GAL1 overexpression (OE) or control vector (CV) lentivirus and expression was confirmed via qPCR, western blot (WB), and flow cytometry. Cell proliferation (doubling time), viability and bioenergetics were assessed using Trypan Blue, CellTiter-Glo, and Seahorse Assay then compared via t-tests. Sambucus nigra lectin (SNA) pull down, which recognizes and binds ST6GAL1-mediated sialylation sites, was utilized to assess for sialylation of GLUT1. Immunofluorescent (IF) staining of surface GLUT1 was performed. Our results confirmed increased ST6GAL1 protein and surface sialylation in our CT26 ST6GAL1 OE cells. OE cells exhibited a shorter doubling time (0.32 days vs. 0.60 days for OE vs CV, $p = 0.03$) and increased proliferation (1.74-fold change; $p = 0.03$). When metabolism was assessed via Seahorse Assay, significantly increased compensatory glycolysis rates versus CV cells ($p = 0.001$, $n = 3$) were identified. In addition, when rotenone was added to block mitochondrial respiration via complex 1 and cells were assessed via CellTiterGlo, we found a decrease in luminescence of both cell types, however, OE cells exhibited a significant attenuated response to the inhibition compared with CV cells (β: 0.15, 95% CI: 0.05 - 0.24, $p = 0.003$). Mass spectrometry and WB identified sialylated GLUT1. IF revealed increased cell surface GLUT1 on OE cells compared to CV ($p = 0.04$). In conclusion, CT26 ST6GAL1 OE cells have accelerated in vitro proliferation and altered cellular metabolism. We found that GLUT1 is sialylated and increased on the cell surface in addition to the ST6GAL1 OE cells possibly leading to the alteration in metabolism seen in these cells. ST6GAL1-mediated GLUT1 sialylation appears to cause increased CRC cell growth and metabolic reprogramming. Additional studies are warranted to further understand the mechanism of altered metabolism.

(P-043) Expression of highly-sulfated keratan sulfate in papillary thyroid carcinoma

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Background: Highly sulfated keratan sulfate (KS) is frequently detected at elevated levels on the cell membranes of papillary thyroid carcinoma (PTC) cells compared to normal thyroid tissue. However, the relationship between the presence of KS and carcinogenesis, as well as its association with common PTC mutations (e.g., BRAF), remains unclear. In other types of thyroid cancer, such as follicular thyroid carcinoma, KS expression is reduced. This suggests that KS may, at least in part, contribute to the slow proliferation characteristic of PTC. Additionally, the glycan structure of thyroglobulin, which increases in the presence of thyroid cancer, remains incompletely understood. Objective: To improve the diagnostic accuracy of PTC through immunohistochemical analysis of thyroid, thymus, and lymph node tissues using a novel anti-sulfated KS antibody. Methods: We previously developed a monoclonal antibody, 299-1C1, which recognizes sulfated KS. Quantitative immunohistochemical analysis was performed on surgical specimens from PTC patients ($n = 50$) to determine the Allred score. The staining patterns and intensities of 299-1C1 were compared with those of

established antibodies 5D4 and R-10G. To identify the epitope of 299-1C1, we conducted immunofluorescence staining using CHO cell lines engineered to express various forms of sulfated KS. Results: PTC tissues stained with 299-1C1 showed significantly higher Allred scores than those stained with 5D4 or R-10G. Both 299-1C1 and 5D4 were found to recognize sulfated galactose epitopes. Interestingly, 299-1C1 also stained thymus and lymph node tissues that were not histopathologically diagnosed as metastatic PTC, suggesting potential utility in identifying microscopic or early metastatic lesions. Conclusion: The novel monoclonal antibody 299-1C1 demonstrated significantly stronger immunoreactivity in PTC tissues compared to other KS antibodies. These findings suggest that 299-1C1 may be a valuable tool for detecting PTC and potentially its metastatic lesions.

(P-044) HNK-1-Capped PTPRZ as a Diagnostic Biomarker and Therapeutic Target in Glioma

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Gliomas are the most prevalent primary tumors of the central nervous system (CNS). Despite advances in imaging, neurosurgical techniques, and radiotherapy, effective treatment of high-grade gliomas remains challenging. The lack of reliable and clinically applicable biomarkers further complicates their diagnosis. In particular, imaging alone is often insufficient to distinguish gliomas from multiple sclerosis (MS) and primary central nervous system lymphoma (PCNSL), necessitating invasive biopsy.Protein tyrosine phosphatase receptor type Z (PTPRZ) is highly expressed in gliomas and is extensively modified with multiple classes of glycans, including chondroitin sulfate, keratan sulfate, N-glycans, GalNAc-type O-glycans, and O-Man glycans. Notably, PTPRZ carry the brain-specific human natural killer-1 (HNK-1) epitope on core M2 glycans generated by the branching enzyme GnT-IX (MGAT5B). We previously found that soluble PTPRZ (sPTPRZ), generated by proteolytic shedding of PTPRZ, is elevated more than tenfold in the cerebrospinal fluid (CSF) of patients with glioma compared with controls. Because extensive glycosylation of CSF sPTPRZ limits its detection by commercially available anti-PTPRZ antibodies, we developed a first-generation GlycoCapture-sPTPRZ assay using monoclonal antibodies against the highly antigenic HNK-1 epitope for both capture and detection.The first-generation assay employed recombinant sPTPRZ engineered to carry HNK-1 glycans as a standard, which resulted in lot-to-lot variability. We therefore established a second-generation GlycoCapture-sPTPRZ assay using a complex of synthetic biotinylated HNK-1 glycans and streptavidin as a standardized calibrator. CSF sPTPRZ levels were significantly elevated in patients with glioma compared with both controls and patients with PCNSL, enabling robust discrimination between these groups (AUC = 0.910 vs controls; AUC = 0.805 vs PCNSL; $p = 0.0010$). Approximately 30% of glioma samples were negative for sPTPRZ. Although PTPRZ-MET gene fusions may account for a subset of these cases, the molecular basis underlying the remaining sPTPRZ-negative tumors is currently under investigation.We further investigated the functional significance of PTPRZ glycosylation using a glioma xenograft model. CRISPR-mediated disruption of either PTPRZ or GnT-IX (MGAT5B) in human LN-229 glioma cells markedly suppressed tumor growth, indicating that PTPRZ and its HNK-1-capped core M2 glycans contribute to glioma progression.Together, these findings identify HNK-1-capped glycosylation of PTPRZ as a glioma-associated molecular signature with both diagnostic and therapeutic potential. The GlycoCapture-sPTPRZ assay may provide a minimally invasive approach for distinguishing diagnostically challenging gliomas and may also have utility for monitoring therapeutic responses, while the PTPRZ–GnT-IX–HNK-1 glycosylation axis represents a potential therapeutic target in glioma.

(P-045) E-selectin Ligand Expression by Acute Myeloid Leukemia Activates Survival Pathways and Affords Chemotherapy Protection

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E-selectin ligands display sialyl Lewis a/x (SLea/x) epitopes and mediate tethering to vascular endothelium. In contrast to the peripheral vascular endothelium, E-selectin is constituently expressed in the protective niches in the bone marrow vasculature where it functions to help produce blood. Blasts and leukemia stem cells from patients with acute myeloid leukemia (AML) exhibit

functional expression of E-selectin ligands (SLea/x), as demonstrated by flow cytometry analysis. Consequently, AML cells bind to E-selectin and can immobilize in the bone marrow. Mean fluorescence intensity of E-selectin-Fc binding was 4-fold higher for relapsed/refractory patients than for newly diagnosed patients (p=0.0026), suggesting that sequestration in the vascular niche of the marrow is associated with chemotherapy resistance. Percent E-selectin-Fc binding was higher in patients with unfavorable than favorable/intermediate risk (p=0.019). Binding of AML blasts to immobilized E-selectin ligand activated Hedgehog and Wnt survival pathways. Use of the small molecule inhibitor GMI-1271 in immunodeficient mouse xenografts bearing human AML demonstrated improved survival when added to daunorubicin and cytarabine chemotherapy. These studies suggest that targeting E-selectin may be effective in improving survival in AML treated with combination chemotherapy.

(P-046) Glycan-Directed Chemoenzymatic Synthesis of Homogeneous Antibody–Drug Conjugates with Tunable Drug Loading

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Antibody Drug Conjugates (ADCs) are an important class of anti-cancer therapeutics that leverages the high specificity of an antibody to selectively deliver a potent cytotoxic payload to tumor cells. The therapeutic efficacy and safety depend critically on the site of conjugation and the drug-to-antibody ratios (DAR). The conserved Fc N-glycan site on IgG1 provides an attractive conjugation site for cytotoxic payloads as it preserves the antigen binding properties and avoids antibody protein engineering. In this study, we report a chemoenzymatic glycoengineering strategy for the construction of Fc-glycosite-specific ADCs carrying two, four, six and eight drugs per antibody molecule. We achieved this by using an E. coli expressed Y285L mutant of human □-1,4-galactosyltransferase 1 (B4GalT1) to transfer the clickable N-azidoacetylgalactosamine (GalNAz) moiety which allows cytotoxic drug conjugation via strain-promoted azide-alkyne cycloaddition (SPAAC) reaction. DAR2 and DAR4 ADCs were constructed using the deglycosylated and native biantennary glycan respectively. We employ the unique ability of the glycosynthase mutant Endo-F3 D165A, from Elizabethkingia meningoseptica, to transfer triantennary glycan oxozaline to construct the DAR6 ADC. For the preparation of DAR8 ADC, we utilized the broad substrate specificity of Pd2,6ST, an α-2,6-sialyltransferase, from Photobacterium damselae, to transfer SiaNAz (N-azidoacetalneuraminic acid) to the GalNAz acceptors on the biantennary antibody glycoform. The cell viability studies showed that all the ADCs exhibited potent cytotoxicity against antigen-positive cancer cells, and the potency appeared to be proportional to the DAR numbers. The present study establishes a facile and novel approach to construct glycosite-specific antibody-drug conjugates with tunable DAR numbers.

(P-047) The participation of the polypeptide N-acetylgalactosaminiltransferase 6 (pp-GalNAc-T6) and O-linked glycans in the drug resistance phenotype in breast cancer cells.

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Cancer cells exhibit changes in the glycosylation pattern of glycoproteins due to alterations in the expression and activity of enzymes involved in the biosynthesys of glycoconjugates, such as glycosyltransferases. Multidrug resistance (MDR) remains a major obstacle to effective chemotherapy, one of the main cancer treatment modalities. Several studies indicate that the emergence of the MDR phenotype is accompanied by changes in the activity and/or expression of glycosyltransferases. Polypeptide-N-acetylgalactosaminyltransferase 6 (pp-GalNAc-T6) is the main enzyme described to date as potential candidate for the synthesis of oncofetal O-glycosylated fibronectin (onf FN), an isoform of FN found in cancer cells and embryonic tissues. Furthermore, the participation of this enzyme, as well as onf FN, has never been investigated in the context of the MDR phenotype in breast cancer cells. Therefore, the objective of this work is to investigate the expression of pp-GalNAc-T6 in MDR breast cancer cell lines and its participation in the byosynthesis of FN onf and in the acquisition of the MDR phenotype. First, an MDR breast cancer cell model was established by treating the MCF-7 cell line with sublethal concentrations of 5, 10, 20, and 40 nM of doxorubicin (DOX) for 6 months. Subsequently, the MTT assay was repeated, and it was observed that cells treated with DOX showed an increase in IC50 for both DOX and the chemotherapeutic agents vincristine (VIN) and cisplatin (CIS). This was accompanied by increased expression

of the efflux pumps AGCG2 and ABCC1 and the anti-apoptotic proteins Bcl-2 and Bcl-xL. Therefore, it can be concluded that treatment with DOX induced the emergence of the MDR phenotype. Concomitant with the emergence of the MDR phenotype, alterations in the extracellular glycophenotype of these cells were detected, such as an increase in the biosynthesis of the O-linked glycan called the Tn antigen, which, when present in the hexapeptide VTHPGY located in the III-CS domain of the FN, originates the smallest epitope of an onf FN. Furthermore, these cells showed increased expression of pp-GalNAc-T6 and onf FN. This led us to hypothesize that pp-GalNAc-T6 performs the biosynthesis of onf FN and participates in the maintenance of the MDR phenotype. To confirm this hypothesis, pp-GalNAc-T6 was silenced and its impact on the MDR phenotype was analyzed. Silencing pp-GalNAc-T6 reduced the expression of FN onf, the BcL-2 and BcL-xL and the IC50 for DOX, VIN, and CIS. Taken together, our results demonstrate for the first time the upregulation of O-glycosylated oncofetal fibronectin, as well as the direct participation of pp-GalNAc-T6 during the acquisition of the MDR phenotype in a breast cancer model, lending credence to the hypothesis that, in cancer cells, glycosyltransferases and/or their products, such as specific isoforms of extracellular matrix glycoproteins, can be used as potential therapeutic targets for cancer treatment.

(P-048) ST6GAL1 containing extracellular vesicle release is increased after chemoradiation in rectal cancer cell lines

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Colorectal cancer (CRC) is the third most prevalent form of cancer in the US with younger Americans increasingly comprising a larger fraction of these cases. Rectal cancers comprise about 1/3 of all CRC cases. In rectal cancer the standard of care is chemoradiation therapy (CRT) accompanied by surgical resection where necessary. Approximately 25% of CRT patients will show a complete response to treatment demonstrating that treatment resistance in rectal cancer remains a clinical challenge. We have previously shown that a sialyltransferase called ST6GAL1 is upregulated in CRC cell lines, has the capacity to transfer treatment resistance in vitro, and correlates with poor response to CRT in patients. It has also been shown that ST6GAL1 is packaged into extracellular vesicles of several cancer cell lines. Previously we reported that the ST6GAL1 in these isolated ECVs is successfully taken up and internalized by cells and retains its biological ability to confer treatment resistance in vitro. LGR5 is a cancer stem cell marker that has been shown to promote cell proliferation and survival. Stem cells enriched in LGR5 have also been shown to have elevated ECV release. Taken together we hypothesized that CRC cell lines would release more ST6GAL1 containing ECVs following chemoradiation and that cells enriched in LGR5 would produce more ECVs compared to LGR5 deficient cells.Using the CRC cell lines SW620 and RKO we isolated and validated the ECVs produced by these cell lines using Western blotting, TEM, and Nanosight. In addition, we performed Nanoimager analysis to evaluate if these ECVs contain α-2,6 sialic acid on their surface and to further confirm these ECVs contain canonical ECV biomarkers. Using our RKO and SW620 cell lines, we treated both cell lines with 5 Gy of CRT, isolated ECVs, and quantified them using nanoparticle tracker analysis (NTA) normalizing particle concentrations to total cell count. Using lentiviral transduction, we engineered an SW620 KD cell line and performed co-culture using SW620 CV and KD cells to further assess the capacity to transfer resistance in vitro. We performed flow cytometry to sort and then separately cultured LGR5 (+) and LGR5 (-) cells, isolated ECVs from both populations, and quantified with NTA.CRT resulted in an increased release of ECVs/cell compared to control in both SW620 and RKO (SW620: $p=0.0001$, $n=9$ and RKO: $p=0.044$ $n=4$). Co-culture of SW620 KD cells with CV cells showed that resistance can be transferred when media is shared between cells ($p=0.013$, $n=5$), further validating our previous findings when we treated with exogenously added ECVs. Our preliminary results show that LGR5 (+) cells release approximately 21 times more ECVs than LGR5 (-) cells ($p=0.00351$, $n=3$). Taken together ECVs released by CRC cell lines transfer resistance in vitro and CRT increases the release of these ECVs. These ECVs are more abundant in LGR5 enriched CRC cells.

(P-049) Glycan as a metabolic driver of pediatric acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, with overall survival rates approaching 90%. However, survival following relapse plummets to approximately 50%, highlighting the urgent need for new therapeutic strategies

for relapsed disease. The hyperactive NSD2-E1099K mutation is frequently acquired at relapse despite being rare at diagnosis, suggesting that NSD2-driven epigenetic reprogramming confers a selective advantage during disease progression. Here, we identify aberrant glycan metabolism as a metabolic vulnerability associated with NSD2-E1099K ALL. Transcriptomic analyses revealed significant upregulation of glycan metabolic pathways in NSD2-E1099K cells compared with an isogenic cell line in which NSD2 was reverted to wild type. Spatial metabolomics further supports these findings with increased glycan abundance in NSD2-E1099K ALL cells. Notably, glycan accumulation was also observed within the meninges, a major site of central nervous system (CNS) relapse in ALL, suggesting that altered glycan metabolism may contribute to CNS disease progression and relapse. Together, these findings identify glycan metabolism as a previously unrecognized metabolic feature of NSD2-mutant ALL and suggest that targeting glycan metabolism may represent a promising therapeutic strategy for relapsed disease.

(P-050) Inhibition of ST6Gal2 Suppresses Tumorigenic Behavior in Neuroblastoma Cells

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Neuroblastoma (NB) is the most common extracranial solid tumor of childhood, comprising 7% of pediatric malignancies but accounting for ~15% of childhood cancer deaths. NB is heterogeneous, with high-risk disease behaving most aggressively. Sialyltransferases attach sialic acid to cell-surface glycoproteins in a process called sialylation. ST6Gal2, a sialyltransferase enriched in neural tissue, attaches sialic acid in an alpha2,6-linkage, a modification also produced by ST6Gal1, an established oncogene in several adult cancers. In NB patients, elevated ST6GAL2 expression correlates with worse overall and event-free survival, advanced stage, and MYCN amplification, a marker of poor prognosis. We hypothesize that ST6Gal2-driven alpha2,6-sialylation enhances viability, proliferation, migration, and invasion in high-risk NB via downregulation of known tumorigenic pathways.We used two high-risk NB cell lines: SK-N-AS (MYCN non-amplified) and SK-N-BE(2) (MYCN amplified). Each was transduced via lentivirus with one of three ST6GAL2-targeting shRNAs (shRNA1-3) or a non-targeting shControl for stable knockdown. Knockdown was verified by Western blot and the shRNAs yielding the greatest knockdown (shRNA2 and shRNA3) were used for functional assays. Expected reductions in alpha2,6-sialylation were confirmed by Sambucus nigra agglutinin flow cytometry. Viability/proliferation were assessed by cell counts with trypan blue exclusion, migration by scratch and Transwell assays, and invasion by Transwell assay, with images quantified in FIJI and experiments performed in triplicate. RNA-seq was performed and analyzed using Plasmidsaurus. Groups were compared by Student's t-test, with mean ± standard error of the mean reported and p<0.05 considered significant.In SK-N-AS cells, ST6GAL2 knockdown increased cell death ~4-fold and reduced proliferation by ~75% by 72 hours (p<0.0001), with impaired scratch-wound closure at 48 hours (p<0.0001). In SK-N-BE(2) cells, knockdown reduced viability by ~12%, decreased total cell number by ~50% at 96 hours, and reduced migration by ~45% (p<0.05 for all). RNA-seq of ST6GAL2 knockdown in both cell lines revealed broad suppression of tumorigenic and plasticity programs (EMT, KRAS signaling, apical junction), cell fate/differentiation pathways (apoptosis, P53 signaling, Notch signaling), inflammatory/cytokine pathways (TNF-α/NF-κB, IL-6/JAK/STAT3, IL-2/STAT5), stress adaptation pathways (hypoxia, reactive oxygen species), and glycolytic metabolism.ST6Gal2-driven alpha2,6-sialylation contributes to tumorigenic behavior in high-risk NB regardless of MYCN status, establishing its relevance to disease progression. These data support further evaluation of ST6Gal2 as a therapeutic target across NB subtypes, with future work needed to define the underlying molecular pathways and specific glycoprotein targets.

(P-051) The role of glyco-redox interplay with FUT8 and ferroptosis in pancreatic cancer cells

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Glyco-redox has yielded new insights into glycobiology and redox regulation1; while further research is needed, it has the potential to open up new avenues for elucidating the mechanisms underlying disease onset. Pancreatic cancer is refractory and the five-year survival rate remains at around 10% worldwide. Chemotherapy and immunotherapy have an inadequate response due to the stroma-rich tumor microenvironment, a major feature of pancreatic cancer. This leads to the rapid development of drug-resistant cells during chemotherapy. One way to combat this resistance would be to make pancreatic cancer cells vulnerable to forms of cell death other

than apoptosis, such as ferroptosis, necroptosis, or pyroptosis. Ferroptosis is being explored as a means of overcoming drug resistance because pancreatic cancer cells produce high levels of reactive oxygen species (ROS).Specifically, the upregulation of FUT8 has been well documented in several cancers, including pancreatic cancer. Several studies have indicated that FUT8 promotes invasion and is associated with pancreatic cancer metastasis. Therefore, FUT8 is a factor that promotes pancreatic cancer progression. In this study, we discovered that FUT8 plays a role in resisting ferroptosis in PANC-1 pancreatic cancer cells. FUT8 knockout (KO) did not drastically affect cell growth. Interestingly, however, FUT8 KO was sensitive to two classes of ferroptosis inducer: (1S, 3R)-RSL3 and imidazole ketone erastin (IKE). Under IKE treatment, FUT8 KO cells exhibited approximately three times the level of lactate dehydrogenase release compared to the wild type (WT). Adding sufficient concentrations of ferroptosis inhibitors such as ferrostatin-1 (for lipid peroxidation), deferoxamine (for iron chelation) and N-acetylcysteine completely cancelled out this sensitivity. Basal lipid ROS levels increased by approximately 1.2- to 1.4-fold in FUT8 KO cells. This suggests that hydroxyl radicals, lipid radicals and lipid peroxide radicals accumulated in FUT8 KO cells at basal levels. Furthermore, FUT8 KO cells showed a 1.4-fold increase in lipid ROS levels compared to WT IKE-treated cells. These results suggest that FUT8 KO affects lipid ROS levels in both basal conditions and those inducing ferroptosis. Therefore, FUT8 KO may alter ferroptosis vulnerability by affecting the ferroptosis-related pathway. Additional inducer treatment can efficiently trigger the ferroptosis of FUT8 KO cells. While the molecular target of FUT8 and the underlying mechanisms are still under investigation, these results suggest that FUT8 plays a role in conferring some degree of resistance under ferroptosis-inducing conditions. This knowledge needs more extensive studies in relation to glyco-redox approach.Reference1.Taniguchi, N., Ohkawa, Y., Kuribara, T., Abe, J., Harada, Y., Takahashi, M. Antioxid Redox Signal. 2024, 41, (13-15), 910-926.

TOPIC 5: Cardiovascular, Hematologic, and Metabolic Glycobiology

(PT-007) O-GlcNAcylation Regulates ACTH Secretion in Corticotroph Adenoma Models

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Cushing's disease is a major cause of endocrine-associated secondary hypertension and is characterized by adenomatous growth of pituitary corticotroph cells that secrete excess adrenocorticotropic hormone (ACTH). Elevated ACTH drives chronic hypercortisolism, leading to significant cardiovascular and metabolic morbidity. O-linked β-N-acetylglucosamine (O-GlcNAc) is a dynamic intracellular glycosylation that couples nutrient sensing to transcriptional and post-transcriptional regulation; however, its role in pituitary hormone secretion remains incompletely understood. Here, we investigated the contribution of O-GlcNAcylation to ACTH production and secretion in corticotroph adenoma models.To define the role of O-GlcNAc signaling in corticotroph function, we utilize the murine corticotroph adenoma cell line AtT-20, mouse models with genetic disruption of O-GlcNAc cycling enzymes, and primary human pituitary cultures derived from surgical resections. Pharmacologic reduction of O-GlcNAcylation decreases ACTH secretion in AtT-20 cells and human corticotroph cultures. Conversely, genetic disruption of O-GlcNAc-regulating enzymes, associated with elevated O-GlcNAc levels, within the pituitary of our mouse models result in elevated circulating stress hormone in vivo. These findings are consistent with our previous studies demonstrating O-GlcNAcylation promotes the stability of pro-opiomelanocortin (POMC) mRNA, the precursor transcript encoding ACTH.Collectively, our results identify O-GlcNAcylation as a critical regulator of corticotroph hormone production and secretion. These findings expand the role of O-GlcNAc signaling in endocrine regulation and suggest that targeting O-GlcNAc-dependent pathways may represent a novel therapeutic strategy for ACTH-driven morbidities in Cushing's disease.

(PT-008) Nascent pre-platelets and B4GALT1 glycosylation contribute to 5-FU–induced bone marrow recovery

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The bone marrow (BM) is a three-dimensional network of branching sinusoids surrounding hematopoietic cells, embedded in a glycan-rich extracellular matrix (ECM) essential for cell maintenance and function. While megakaryocyte (MK)-environment interactions during BM recovery are increasingly appreciated, the role of glycan-dependent thrombopoiesis and nascent platelets in BM maintenance and reconstitution remains poorly understood. We previously showed that loss of β-1,4-galactosyltransferase 1 (B4GALT1) causes accumulation of hyperactive β1 integrin, aberrant MK and platelet adhesion to ECM ligands and thrombocytopenia, but its role in BM recovery after chemotherapeutic injury (5-fluorouracil, 5-FU) is unknown.Using a 5-FU injury model in B4GALT1-deficient ($B4^{-/-}$) mice, we tracked BM recovery over 10 days following a single dose of 5-FU (150 µg/g). Using high resolution BM imaging and intravital microscopy, we analyzed MKs number, distribution, ECM markers and nascent platelet deployment within the BM niche. Flow cytometry was used to evaluate platelet activation MK counts and hematopoietic stem and progenitor cells (HSPCs). Following 5-FU treatment of control mice, we identified a transient intramedullary space enriched in ECM proteins together with BM-resident platelet-like cells expressing canonical platelet markers and exhibiting robust activation upon collagen related peptide stimulation (termed pre-platelets). This intramedullary space requited approximately 8 days for complete resolution. Platelet depletion before 5-FU administration significantly delayed this process, implicating newly generated platelets in BM recovery following 5-FU. $B4^{-/-}$ mice exhibited persistent thrombocytopenia and failed to reconstitute circulating platelet counts post–5-FU treatment. These mice also displayed morphologically abnormal, dysplastic MKs, altered MK spatial localization, and expansion of MK-biased HSPCs following 5-FU injury. Collectively, these abnormalities were associated with delayed hematopoietic recovery and persistence of the intramedullary space, suggesting that efficient platelet production and MK integrity contribute to BM recovery after 5-FU.To determine whether these effects were specifically mediated by B4GALT1 in MKs, we crossed C57BL/6J B4galt1fl/fl with Pf4-Cre mice to generate MK/platelet-specific B4galt1-deficient mice (B4MK-/-). Consistent with the global knockout, these conditional mice recapitulated the thrombocytopenia phenotype and showed a two-fold increase in mature (≥8N) MKs ($p=0.02$). Unexpectedly, we also observed a 3.5-fold increase in 2N, low-ploidy MKs ($p=0.01$), indicating a bimodal disruption of MK ploidy, both an excess of terminally mature MKs and an accumulation of immature, low-ploidy MKs, pointing to impaired MK maturation dynamics following MK-specific loss of B4GALT1.Together, these findings identify B4GALT1-dependent glycosylation as a key regulator of MK maturation, thrombopoiesis and HSC homeostasis.

(P-052) Blood glycan heterogeneity in healthy and disease human populations

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The glycocalyx is a 10–100 nm glycan-rich shell that covers all blood cells. In addition to their roles in development, these complex carbohydrates regulate immune cell function. Although selected aspects of blood-cell glycosylation have been examined, methods to comprehensively characterize them on normal and diseased human blood cells at single-cell resolution remain limited. To address this gap, we developed SpecLec, a spectral flow cytometry–based lectin-profiling method to measure N- and O-linked carbohydrate epitopes across approximately 35 immune cell types using a panel of 23 carbohydrate-binding lectins. Computational inference enabled annotation of glycan motifs across blood-cell populations. In peripheral blood, each cell type displayed a characteristic glycan profile, with shared signatures among populations related by function or ontology. For example, T cell populations showed high MALII binding, whereas NK cells and cytotoxic T cells shared low RCA engagement. In bone marrow, glycans shifted progressively as cells underwent lineage commitment and terminal differentiation. Lectins such as GSLII and VVA showed divergent expression patterns between myeloid and lymphoid lineages. Finally, we identified multiple glycan signatures that differed between normal bone marrow cells and diseased bone marrow cells from a TP53-dependent myelodysplastic syndrome patient cohort. In particular, VVA and WGA binding were prominently dysregulated among cytotoxic T cells and other lineages, in addition to hematopoietic stem and common myeloid progenitors. These data suggest that lesions in the marrow microenvironment may systemically alter glycan structures across the immune compartment. All data are made available through an accompanying web portal. Together, this study provides a first draft of a human blood glycan atlas.

(P-053) Reduced Oligosaccharyltransferase Complex and N-Glycoproteins in Human Heart Failure: A Cross-Cohort Analysis

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Background: N-linked glycosylation regulates protein folding, trafficking, quality control, and cell-surface signaling—processes essential for cardiomyocyte function. Cardiomyocyte-restricted disruption of complex N-glycan biosynthesis has been reported to cause heart failure with reduced ejection fraction (HFrEF) in mice. However, upstream alteration of N-glycosylation machinery in human myocardium remains unknown. We investigated whether endoplasmic-reticulum oligosaccharyltransferase (OST) complex subunits and candidate downstream N-glycoprotein substrates show recurrent alterations across human HF phenotypes.Methods: Seven publicly available human myocardial proteomic datasets (PXD060431, PXD033876, PXD043768, PXD042155, PXD008934, PXD012664, and linked PXD014826/PXD018678 cohorts; n=117 HF, n=84 controls) spanning HFpEF, advanced HFrEF, dilated cardiomyopathy (DCM), and ischemic cardiomyopathy (ICM) were analyzed. Reported abundances were log-transformed and z-scored within cohort. Cross-cohort analysis focused on OST catalytic subunits STT3A/STT3B and associated components RPN1, RPN2, DDOST, and MAGT1. Group differences were assessed using Welch's t-test. Dysregulated proteins were cross-referenced against experimentally identified N-glycoproteins from published cardiac and multi-tissue glycoproteomic studies and evaluated for pathway enrichment using Enrichr.Results: Consistent reductions in OST subunit abundance were most evident in HFpEF and advanced HFrEF myocardium. HFpEF showed decreased abundance of all six OST components (STT3A, STT3B, RPN1, RPN2, DDOST, and MAGT1), with log2 fold changes ranging from −2.94 to −1.58. STT3A, STT3B, and RPN1 remained significantly reduced after multiple-testing correction (adjusted P=0.002–0.028). Advanced HFrEF left ventricular myocardium independently showed concordant decreases in all six OST components. DCM and ICM cohorts showed partial and phenotype-dependent replication, with RPN1 emerging as the most consistently decreased OST component across all four phenotypes. HF-dysregulated proteins with experimentally supported N-glycosylation showed enrichment for SRP-dependent cotranslational protein targeting to membrane, neutrophil degranulation, neutrophil activation, complement and coagulation cascades, and phagosome pathways ($q \leq 8.82 \times 10^{-4}$).Conclusions: Cross-cohort analysis of human myocardial proteomics identifies recurrent, phenotype-dependent reductions in OST subunit abundance, most consistently involving RPN1, together with enrichment of dysregulated N-glycoproteins in protein-targeting and immune/inflammatory pathways. Proteomic abundance does not report catalytic activity or site occupancy; these data nominate the OST–N-glycoprotein axis as a candidate molecular pathway warranting site-specific glycoproteomic validation.

(P-054) Knockdown of Fringe protein expression represses osteoclast activity

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Osteoporosis is a significant source of morbidity within the United States, affecting about 12.6% of the population. Disproportionate and excessive activity of osteoclasts, which are the multinucleated cells responsible for bone resorption during physiologic bone remodeling, underpins the development of osteoporosis. At the molecular level, Notch signaling is an important modulator of osteoclastogenesis and osteoclast function. Additionally, Fringe proteins regulate Notch signaling by attaching N-acetylglucosamine to fucose sugars o-linked to the extracellular domain of Notch receptors. There are three Fringe protein homologs – Lunatic, Manic, and Radical. Each uniquely alters Notch receptors, either enhancing or diminishing Notch signaling in a receptor- and ligand-selective manner. While all three Fringe proteins are expressed throughout osteoclastogenesis, Lunatic expression dominates. For our work, we hypothesized that the Fringe proteins, especially Lunatic, establish baseline osteoclast differentiation. To test this, we used silencing antisense oligonucleotides to systematically knock down each Fringe homolog's expression in murine osteoclast precursors in vitro before differentiating them into osteoclasts. At end point, after confirming knockdown with RT qPCR, we assessed osteoclast morphology and assayed conditioned medium for activity of TRAP, an osteoclast functional enzyme. We found that knockdown of each Fringe homolog corresponds with fewer multinucleated osteoclasts and significant reduction in TRAP activity. To our surprise, even knockdown of the marginally expressed Manic and Radical homologs generated these results. Hereafter we will assess changes in expression of osteoclast functional genes, bone resorption activity, and Notch receptor activation following

Fringe knockdown to further substantiate our findings. This work will define the importance of Fringe proteins for baseline osteoclastogenesis, revealing potential targets for therapeutic strategies to reduce pathologic stimulation of osteoclasts.

(P-055) Hydrogen peroxide mediates high mannose N-glycan expression on red blood cells.

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Recent studies have elucidated a new mechanism involving high mannose (HM)-N-glycan expression on the surface of red blood cells (RBCs) leading to anemia, for example in sickle cell disease. However, the mechanisms regulating HM-N-glycans expression on the RBC surface is unclear. RBCs are constantly producing and / or being exposed to reactive species (e.g. H2O2, HOCl, ONOO- and NO), that often lead to oxidative damage and RBC lysis. In this study, human RBCs were exposed to a variety of oxidants and only H2O2, under conditions that did not cause hemolysis, increased HM-sugars observed by FACS using ConA or HHL lectin; controls groups involving co-incubation with mannose confirmed HM-expression. Surface expression of HM-epitopes occurred relatively quickly (within 5min of H2O2), with expression decreasing over 30min. Pre-treatment of RBCs with carbon monoxide to block ferrous hemoglobin attenuated H2O2 dependent increases in HM-expression suggesting a key role for heme/redox cycling reactions. Human monocyte derived macrophage dependent erythrophagocytosis was higher with RBCs treated with H2O2 which was prevented by co-incubation with Mannan supporting a role for surface HM epitopes in RBC clearance. Finally, we observed higher level of surface HM-sugars on RBCs collected from sickle cell patients at baseline and after H2O2 treatment. Together these data suggest a role for H2O¬2, independent of hemolysis, in modulating RBC turnover by surface HM-dependent erythrophagocytosis.

(P-056) GNE-dependent Sialylation is Required for Megakaryocyte Maturation and Platelet Function

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Thrombocytopenia remains a major and frequently life-threatening condition seen in infection, autoimmunity, and myelosuppressive therapy. Current treatments are largely supportive and often rely on platelet transfusions. However, these are limited by short shelf life, variable efficacy, persistent shortages, and failure to address underlying defects in platelet production or function. Defining the molecular pathways governing platelet homeostasis is essential to developing mechanism-based therapies. Sialylation, the terminal addition of sialic acid (Sia) to cell-surface glycans, mediates self-recognition and regulates immune cell trafficking, survival, and signaling through Siglec (sialic acid-binding immunoglobulin-type lectin) receptors. Loss of platelet-surface Sia triggers rapid hepatic lectin-mediated clearance, governing platelet lifespan and count. Human genetics underscores this pathway's clinical significance: variants in GNE, the rate-limiting enzyme in Sia biosynthesis, cause severe thrombocytopenia, and a growing number of pediatric case reports now link congenital thrombocytopenia to GNE variants. While Sia's role in platelet survival is well established, its function in megakaryocyte (MK) differentiation, maturation, and platelet production remains largely undefined, a gap with direct implications for diagnosis and therapy. To test the role of GNE in MK maturation and function, we generated a platelet/MK-specific Gne conditional knockout mouse (Gneplt-/-). Gneplt-/- mice exhibit profound thrombocytopenia with dysplastic MKs, indicating a primary defect in MK maturation rather than peripheral clearance alone. Platelets show impaired collagen receptor function and attenuated activation. Together, these findings establish a MK-intrinsic requirement for GNE-dependent sialylation in megakaryocyte maturation and platelet function and provide mechanistic insights for patients affected by GNE thrombocytopenia.

(P-057) Sex-Specific Regulation of Placental O-GlcNAc Transferase in Response to Maternal Stress

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O-GlcNAcylation is a dynamic post-translational modification essential for placental function and fetal development. In the placenta, it supports trophoblast proliferation and hormone production and is rapidly regulated by the enzymes O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). While most tissues increase global O-GlcNAcylation during stress, pregnancies complicated by gestational diabetes mellitus (GDM) and pre-eclampsia show reduced placental O-GlcNAc levels. These disruptions suggest that altered O-GlcNAc cycling may impair placental adaptation to maternal metabolic stress. One mechanism that may impair placental adaptation is oxidative stress, which is a hallmark of both GDM and pre-eclampsia. Metabolic imbalances lead to increased production of reactive oxygen species (ROS), and when antioxidant defenses are inadequate, oxidative stress is increased. Therefore, we hypothesize that maternal stress increases oxidative stress in the placenta, downregulating OGT, leading to further metabolic dysregulation. This project aims to define the mechanisms that regulate oxidative stress-induced OGT alterations in the placenta. To further define OGT and OGA levels in the placenta, we have expanded our originally published dataset to include subsets of GDM and pre-eclamptic placentas. These data demonstrate downregulation of OGT and OGA in subsets of each condition and is associated with complication severity, particularly in placentas from male fetuses. To test directly if increases in oxidative stress downregulate OGT in the placenta, we exposed placental cell lines and primary human trophoblasts to various oxidative stressors, demonstrating OGT downregulation specifically in male cells. We further show that acute glucocorticoid treatment with cortisol restores OGT expression in male cells after oxidative stress, while chronic glucocorticoid receptor activation via dexamethasone sustains OGT suppression, indicating crosstalk between glucocorticoid signaling and OGT regulation. Because OGT is X-linked and males carry only one allele, we hypothesize that limited compensatory capacity contributes to a heightened vulnerability of male placental cells to oxidative stress. Future studies will directly test whether escape from X-chromosome inactivation in females provides protection. Together, these data provide the first integrated view of how OGT and OGA respond to acute and chronic oxidative stress in placenta, and how these enzymes are altered in pregnancies with metabolic complications.

TOPIC 6: Congenital Disorders of Glycosylation and Rare Diseases

(PT-009) Investigating the Downstream Effects of O-GlcNAc Transferase (OGT) in X-Linked Intellectual Disability

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One percent of the population is affected by an Intellectual Disability (ID), a disorder characterized by a reduction in cognitive and adaptive functioning and an intelligence quotient (IQ) less than 70. Over 1,400 genes have been linked to ID, including over 145 X-chromosome genes, collectively referred to as X-linked Intellectual Disabilities (XLID). The O-GlcNAc transferase (OGT) gene, localized on the X chromosome, encodes the highly conserved nuclear- and cytoplasmic-localized glycosyltransferase that is solely responsible for the addition of O-linked □-N-acetyl-D-glucosamine (O-GlcNAc) from the high-energy UDP sugar donor, UDP-GlcNAc, onto serine and threonine residues of thousands of nucleocytoplasmic proteins. OGT consists of two major domains: an N-terminus consisting of 13.5 tetratricopeptide (TPR) repeats and a C-terminal glycosyltransferase catalytic domain. Missense mutations found in both the TPR and catalytic domains of OGT are linked to XLID (OGT-XLID), affecting more than 60 families. Currently, there is no definitive test to determine if a given mutation is causal for XLID. Here, we utilize CRISPR/Cas9 to genetically engineer a male neuroblastoma cell line, BE(2)-M17, with OGT-XLID knock-in variants to investigate and define mutual downstream impacts. Using RNA sequencing, we are investigating common changes in gene expression. Utilizing mass spectrometry, we are defining shared changes in the O-GlcNAcome. Using proteomics, we are defining any potential conserved secreted biomarkers of the disability. Furthermore, we are utilizing these cell lines to examine the impact of substrate augmentation therapy and pharmacological OGA inhibition as potential interventions for any observed defects. This work will create and

characterize an array of cell lines that will assist us in discovering common downstream impacts in OGT-XLID, as well as providing a platform for developing diagnostic and prognostic biomarkers and therapeutic regimens in cellulo.

(PT-010) COG4-Associated Golgi Dysfunction Impairs Proteoglycan-Rich Matrix Assembly in Patient iPSC-Derived Cartilage Organoids from Saul-Wilson Syndrome

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Abstract:Saul-Wilson syndrome (SWS) is a skeletal dysplasia characterized by primordial dwarfism and caused by a recurrent dominant variant in COG4 (p.G516R), a regulator of Golgi trafficking and glycosylation. Although previous studies demonstrated altered Golgi retrograde trafficking, defective proteoglycan glycosylation, and impaired extracellular matrix (ECM) secretion in cellular and zebrafish models, the developmental mechanisms underlying skeletal dysplasia in SWS remain unclear.Here, we generated patient-derived induced pluripotent stem cell (iPSC) cartilage organoids to investigate the developmental consequences of COG4 dysfunction during human chondrogenesis. SWS organoids exhibited impaired cartilage formation, reduced expression of chondrogenic markers, and defective deposition of proteoglycan-rich ECM. Time-course RNA sequencing revealed diminished activation of transcriptional programs associated with skeletal development, ECM organization, ossification, and glycosaminoglycan metabolism during chondrogenic differentiation. Spatial multiplex CODEX imaging, using a panel of 30+ cell-type markers together with multiple lectins, revealed altered chondrogenic trajectories, persistence of mesenchymal-like cell states, widespread glycosylation abnormalities, and markedly reduced deposition of chondroitin sulfate proteoglycans within developing cartilage.Together, these findings demonstrate that Golgi-associated trafficking defects disrupt ECM glycosylation and proteoglycan assembly during early cartilage development, thereby impairing chondrogenic progression and skeletal growth. This study establishes patient-derived cartilage organoids as a human model for investigating proteoglycan-dependent mechanisms underlying skeletal dysplasia and provides new insight into how defects in Golgi trafficking contribute to abnormal skeletal development in SWS. This work has been supported by the National Institute of Health (Grant R01 DK99551) and The Rocket Fund.

(P-058) Astrocytic Cellular Models of Neurological Glycogen Storage Diseases to Investigate Pre-Clinical Therapeutics

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Pompe disease and Lafora disease (LD) are rare, inherited glycogen storage diseases (GSDs) marked by pathological glycogen accumulation and dysregulated N-glycan metabolism. Recent evidence of central nervous system (CNS) involvement has reclassified both as neurological GSDs (n-GSDs), though the two diseases differ in subcellular localization: in Pompe disease, glycogen accumulates within lysosomes, while in LD it accumulates in the cytosol as polyglucosan bodies (PGBs). Current treatment options are limited. Enzyme replacement therapy is available for Pompe disease, but poor enzyme stability, suboptimal skeletal muscle delivery, and minimal CNS penetration (~0.1% of infused enzyme reaches the brain) restrict its efficacy. Currently, no FDA-approved therapeutic exists for LD. Considering both diseases are rare, a therapeutic capable of targeting multiple subcellular compartments would also make clinical development more financially viable. To address this, we developed astrocytic cellular models of Pompe disease and LD to evaluate subcellular-targeted therapeutics: lysosomal in Pompe disease and cytosolic in LD. Our lab has previously shown that the antibody-enzyme fusion with amylase (AEF-amylase) degrades cytosolic PGBs and corrects N-glycan metabolism in an LD mouse model. Using established Pompe disease and LD primary astrocyte models, we tracked pathological glycogen accumulation over time in each compartment and tested AEF-amylase's ability to degrade PGBs and correct N-glycan metabolism, using biochemical assays, immunostaining, and mass spectrometry. Pathological glycogen accumulated over time in both n-GSD models, with PGBs increasing in size in LD astrocytes, and AEF-amylase successfully degraded these PGBs.

We will next test AEF-amylase's ability to reduce pathological glycogen in the Pompe disease astrocyte model and to correct N-glycan metabolism in both models to establish a preclinical therapeutic strategy capable of treating multiple n-GSDs. These astrocytic n-GSD models and pre-clinical therapeutics provide a tractable system to interrogate glycogen metabolism across cytosolic and lysosomal compartments, and to trace its connection to N-glycan metabolism using isotopic tracing and mass spectrometry-based approaches. Importantly, these astrocytic n-GSD models offer a translational pipeline for evaluating disease-modifying strategies to improve lifespan and quality of life for n-GSD patients.

(P-059) Preclinical Mouse Models of ATP6AP2-CDG Neurological and Hepatological Clinical Manifestations

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Congenital disorders of glycosylation (CDG) are rare multi-systemic genetic diseases caused by disrupted glycosylation. ATP6AP2-CDG was first reported in 2017 as a clinical syndrome with infantile-onset liver failure, neurological impairment, immunodeficiency, and cutis laxa. ATP6AP2 is a component of the vacuolar-type ATPase, which functions to maintain vesicular pH, such as in the Golgi apparatus and lysosomes. There is currently no targeted therapy for ATP6AP2-CDG. Here, we sought to generate preclinical mouse models of major clinical manifestations of ATP6AP2-CDG to study disease pathophysiology and evaluate novel therapeutic approaches to disease treatment. We generated conditional ablation of Atp6ap2 in neurons and hepatocytes using an available floxed mouse to target primarily affected organs in ATP6AP2-CDG. Using the Snap25-Cre mouse line to conditionally ablate Atp6ap2 in post-mitotic neurons resulted in affected mice showing a decreased body mass, an abnormal gait and hind limb clasping, and neuronal degeneration. Affected mice exhibited abnormal brain glycosylation and lysosomal accumulation. Notably, protein levels of lysosomal markers such as Lamp1 and Cathepsin D were upregulated in cortical samples of affected mice. We also identified glycomic disruption in affected cortex. Using the Albumin-Cre mouse line to conditionally ablate Atp6ap2 in hepatocytes resulted in abnormal elevations in liver markers such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), as well as cholesterol. Affected mice exhibited abnormal serum and liver glycosylation. Liver histology demonstrated cirrhosis, hepatocyte hypertrophy, and lysosome accumulation. We found an upregulation trend of autophagy and lysosomal factors' protein levels such as Lamp1, Cathepsin D, p62, and LC3I/LC3II ratio in the liver of affected mice. We also identified glycomic disruption in affected liver. In summary, our conditional knock-out mouse models recapitulate key features of neurological and hepatological clinical manifestations of ATP6AP2-CDG, including neurological features (patients exhibit developmental delay, intellectual disability, and seizures) and liver-related abnormalities (patients exhibit elevated transaminases, hepatomegaly, and fibrosis). Thus, we have successfully generated preclinical mouse models of ATP6AP2-CDG for investigation of novel therapeutic approaches for treatment of disease manifestations. Our investigations suggest shared contributions of disrupted glycosylation and lysosomal dysfunction to ATP6AP2-CDG disease pathophysiology. Ongoing investigations include comparison of a knock-in patient mutation, I21M (I22M in mouse), model to our conditional knock-out model for further investigation of disease pathophysiology and novel therapeutic approaches.

(P-060) Two patients with pathogenic variants in the GNE gene present with distinct clinical manifestations

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The GNE gene encodes UDP-N-acetylglucosamine-2-epimerase, a rate-limiting enzyme that regulates the biosynthesis of sialic acid precursors. Sialic acid is essential for the function of muscle fibres and platelet membranes, and abnormalities in the GNE gene can lead to a variety of clinical manifestations. GNE gene abnormalities are known to cause at least three disorders: GNE-related

myopathy (Nonaka myopathy; autosomal recessive [AR]), GNE-related thrombocytopenia (AR), and sialuriroidism (autosomal dominant). However, in the case of myopathy and thrombocytopenia, for example, whilst some patients exhibit both symptoms, others have been found to present with only one of them. The relationship between genotype and phenotype (disease) remains unclear.We report on two patients with pathogenic variants in the GNE gene who presented with different clinical manifestations. Patient 1 was a 13-year-old boy. The chief complaint was persistent thrombocytopenia. His parents were not consanguineous and had no history of thrombocytopenia or haemorrhagic symptoms. Although he presented with purpura, no hearing loss, cataracts, or hepatosplenomegaly were observed. There was no muscle weakness. The results of the neurological examination were normal. His coordinated movement, gait, and autonomic nerve functions were all normal. Coagulation tests revealed a Duke bleeding time of 5 minutes and a von Willebrand factor antigen level of 84 per cent. Patient 2 was a 3-year-old girl. The chief complaint was psychomotor developmental delay. Developmental delay had been noted since infancy. Characteristic facial features were also observed. No significant verbal expression was observed. Hypotonia was observed. No abnormalities were found in general blood tests.After obtaining of written informed consent from the parents, comprehensive genomic analysis was performed. In Patient 1, two novel biallelic variants in the GNE gene (NM_01128227.3), [c.1277del,p.(Asn426IlefsTer13)] and c.1163+5G>T, were identified and confirmed by Sanger sequencing. It was confirmed that the variants were inherited from the father and mother, respectively. In Patient 2, a heterozygous variant [c.527_528del,p.(Ser176fs)] in the GNE gene was identified. The diagnosis for Patient 1 was thrombocytopenia without myopathy. Although Patient 2 presented with clinical findings overlapping with sialouria, this case was considered to represent a clinically distinct disorder. This report is considered to be helpful for understanding the clinical spectrum of GNE-related disorders and the function of UDP-N-acetylglucosamine-2-epimerase.

(P-061) Identification of neurological deficits in PMM2-CDG mouse model recapitulating patient genotype

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Phosphomannomutase 2 deficiency (PMM2-CDG) caused by compound heterozygous pathogenic variants in the PMM2 gene. Individuals with PMM2-CDG exhibit multisystem manifestations, including hepatopathy and neurological dysfunction (most prominently with cerebellar hypoplasia and associated ataxia). However, developing a mouse model of PMM2-CDG has proven challenging due to early embryonic and perinatal lethality and limited recapitulation of neurological phenotypes. A previously reported compound heterozygous R137H/F115L mouse model that recapitulates the most common human patient genotype was studied on a mixed C57BL/6J-129S6/SvEv background and found to exhibit significant embryonic lethality (62% of expected affected offspring fail to survive to birth) followed by a further 25% mortality in the immediate perinatal period accumulating to 50.9% mortality of affected live births by postnatal day 65. Surviving mice failed to reproduce key neurological features of PMM2-CDG. We found that on a pure C57BL/6J background, affected mice exhibit 100% embryonic and perinatal mortality. However, backcrossing onto a 129S6 background substantially improved survivability, and generating mixed C57BL/6J-129S6 F1 compound heterozygous R137H/F115L mice increased the number of mice surviving into adulthood to greater than 90%. Affected mice continued to demonstrate previously reported phenotypes, including stunted growth and protein hypoglycosylation on cell-associated and secreted glycoproteins, including antithrombin III. Given the improved survivability, we report the first evaluation of the model for developmental abnormalities. Initially, we assessed the mice for deficits in early developmental milestones, including pinnae detachment, eye opening, incisor eruption, fur development, surface righting, edge avoidance, negative geotaxis, and grasping reflex and found significant developmental delay in most measures. Subsequently, we assessed adult (~P90) mice with a battery of behavioral assessments, including assessing locomotor activity (open-field and home-cage monitoring), working memory (Y-maze), motor coordination (rotarod and beam walk), and complex task integration (nesting), to identify neurological and motor deficits in adulthood. Compound heterozygous R137H/F115L mice display significant decrease in locomotor activity and deficits in motor coordination. Ongoing studies include glycoproteomic of the adult cerebellum and cortex to identify hypoglycosylated proteins in the brain that may contribute to the neurological dysfunction. This study establishes a critical reference dataset of neurological deficits in a preclinical PMM2-CDG mouse model recapitulating the most common patient genotype for investigating disease pathophysiology and evaluating novel therapeutic strategies, such as adenine base editing to correct the R137H variant, an emerging precision gene-editing approach with potential for in vivo therapeutic application.

(P-062) Mannosyl-oligosaccharide glucosidase (MOGS) deficiency enhances microbially-mediated innate immune sensing in Drosophila

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Mannosyl-oligosaccharide glucosidase (MOGS) is a key enzyme in the N-linked glycosylation pathway that removes the terminal glucose residue from Glc3Man9GlcNAc2 glycan structures in the endoplasmic reticulum to promote protein quality control. Pathogenic variants in MOGS lead to a congenital disorder of glycosylation (CDG) known as MOGS-CDG, whose symptoms include developmental delays, neurological impairments, and immunological abnormalities. However, the underlying mechanisms for MOGS-CDG phenotypes are not known. Here, we show that loss of Drosophila α-glucosidase 1 (GCS1; homolog of human MOGS) results in developmental delay and lethality. One of the predominant phenotypes observed in GCS1-mutant larvae is a significant increase in immune gene expression in the gut and the fat body, an organ similar to the mammalian liver and adipose tissue. Tissue-specific rescue experiments with human MOGS and knockdown studies indicate that GCS1 is required in multiple tissues to promote larval development. Genetic inhibition of the Toll and IMD pathways partially rescues lethality in GCS1 mutants, indicating that excessive immune activation contributes to the phenotype. We further find that microbial exposure is a major driver of immune hyperactivation in GCS1 mutants. Germ-free rearing of GCS1 mutants results in a full rescue of developmental delay, a partial rescue of lethality, and normalization of immune gene expression in the fat body, suggesting that gut dysbiosis contributes to GCS1 mutant phenotypes, including immune hyperactivation in the fat body. However, GCS1 mutant larvae do not exhibit a leaky gut phenotype, suggesting that bacterial immunogens such as peptidoglycan might reach the fat body through hemolymph and induce immune response in this tissue. Indeed, decreasing the gene dosage of a peptidoglycan receptor protein (PGRP-SD) partially suppresses the lethality in GCS1 mutants. Mutant fat bodies exposed to equivalent amounts of peptidoglycan ex vivo exhibit a stronger immune response than controls, suggesting host sensitivity to microbial signals. Further studies are required to confirm the peptidoglycan-mediated immune response induction upon loss of GCS1 and to identify the molecular targets that are prone to altered glycosylation in this mutant background. Dissecting these molecular mechanisms and the modifiers of MOGS/GCS1 loss-of-function phenotypes will provide a better understanding of the pathophysiology of MOGS-CDG and might help us identify potential therapeutic targets for glycosylation disorders.

(P-063) Determining Novel Tissue Specific Regulators of O-mannosylation

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A network of approximately 12 enzymes acts in concert to construct an extended O-mannose glycan (termed functional M3) on α-dystroglycan (α-DG), terminating in the repeating disaccharide matriglycan. Defects in any M3 pathway enzyme cause α-DG hypoglycosylation, underlying a spectrum of congenital muscular dystrophies known as dystroglycanopathies. Beyond muscle degeneration, severe cases present with profound congenital brain and ocular malformations and early mortality. Although numerous causal genes have been identified, approximately 30% of cases are still of unknown genetic etiology. Here we present CRISPR-Cas9 genetic screens aimed at identifying novel gene products involved in the synthesis and regulation of the functional M3 glycan in an effort to elucidate genes that are causal for these unexplained dystroglycanopathies. The screens were executed in a novel MYMK-knockout myoblast line capable of differentiating into myocytes without undergoing fusion. Transcriptomic and proteomic comparisons between wild-type and MYMK-knockout cells demonstrated minimal baseline differences. This cell line enables for the first time single-cell FACS-based Cas9 screening in myocytes that express functional α-DG unlike undifferentiated myoblasts. Utilizing the clinical antibody IIH6, which specifically recognizes matriglycan, we sorted cells that have lost a gene that prevents them from producing the functional M3 glycan on cell surface α-DG. Candidate hits are currently being validated and undergoing functional characterization. Further screens in other disease relevant cell lines such as neural and hepatocarcinoma cell lines are in progress. Finally, the characterized gene list will be evaluated by our clinical collaborators to potentially explain idiopathic cases and uncover new therapeutic targets.

(P-064) Loss of a single high-mannose N-glycan at Asn333 drives ACP3 misfolding, ER retention, and neurodevelopmental disease

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Missense mutations disrupting N-glycosylation motifs can perturb protein folding and trafficking, yet the site-specific contributions of individual N-glycans to proteostasis remain poorly defined. Here, we delineated the functional hierarchy of three N-glycosylation sites in human prostatic acid phosphatase (ACP3). Glycoproteomic analysis showed that Asn94 and Asn220 carry predominantly complex-type N-glycans, whereas Asn333 is enriched in high-mannose structures. Glycan-deficient N94Q and N220Q variants were efficiently secreted and retained phosphatase activity, whereas N333Q and T335M accumulated intracellularly, formed endoplasmic reticulum aggregates, and lacked enzymatic activity. Molecular dynamics simulations revealed that the Asn333 high-mannose glycan forms persistent hydrogen-bond networks with surrounding residues and exhibits markedly reduced conformational flexibility, supporting a non-redundant structural role. Individuals harboring a heterozygous p.T335M variant present severe neurodevelopmental abnormalities, linking Asn333 glycan loss to proteostasis failure in vivo. These findings establish a site-specific glycan–protein stabilizing mechanism with broad implications for glycoprotein folding and human disease.

TOPIC 7: Chemical Glycobiology and Carbohydrate Synthesis

(PT-011) Red Blood Cell Surface Engineering for Influenza Hemagglutination Using Synthetic Sialylated N-Glycans

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Influenza A viruses initiate infection through interactions between hemagglutinin (HA) and sialylated glycans on host cells. Although human- and avian-adapted viruses generally favor α2,6- and α2,3-linked sialic acids, respectively, recognition is also influenced by the underlying glycan architecture, including LacNAc chain length and cell-surface presentation. Conventional hemagglutination assays rely on native red blood cells (RBCs), whose heterogeneous and species-dependent glycomes complicate the interpretation of receptor specificity. Moreover, receptor-binding drifting in emerging influenza strains can reduce agglutination of commonly used RBCs. We therefore sought to develop RBCs displaying chemically defined sialylated glycans as a functional platform for evaluating influenza virus–glycan interactions.A panel of synthetic sialylated N-glycans was prepared to systematically vary three structural features: the terminal sialic acid linkage (α2,3 or α2,6), the symmetrical and asymmetrical N-glycans, and the length of the underlying LacNAc chain. These glycans contain clickable azide and biotin groups so that they were able to be installed on Turkey RBCs using ST6GAL1-intermediated selective exoenzymatic labeling (SEEL), enabling presentation of defined ligands in the multivalent environment of the cell membrane. The resulting engineered RBCs were evaluated in influenza hemagglutination assays using virus preparations that exhibited weak or undetectable agglutination of unmodified RBCs.Installation of the synthetic sialylated N-glycans converted otherwise poorly responsive RBCs into cells capable of robust virus-induced hemagglutination. The gain of agglutination demonstrates that SEEL-installed glycans are accessible to viral HA and function as productive multivalent receptors on the RBC surface. The structurally defined glycan panel further provides a framework for directly comparing the effects of sialic acid linkage and LacNAc extension on influenza recognition.These findings establish glycoengineered RBCs as a bridge between glycan-binding assays and functional virological readouts. Unlike glycan microarrays, the platform presents defined glycan receptors in a native membrane context and directly reports multivalent viralengagement through hemagglutination. It may therefore

facilitate mechanistic studies of influenza receptor specificity and support the adaptation of hemagglutination assays to viral strains that interact inefficiently with native RBCs.

(P-065) Development of Bordetella pertussis Fermentation of Chemically Modified Peptidoglycan Fragments

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The relationship between the human innate immune system and bacterial peptidoglycan (PG) fragments remains to be fully understood due to the high chemical diversity of PG fragments. Tracheal Cytotoxin (TCT) is one of the most potent immunostimulatory PG fragments, yet how it interacts within the immune system has not been fully elucidated. Recently, we observed that mice infected with Bordetella pertussis strain that overproduces TCT, exhibited anti-inflammatory responses in contrast to in vitro models. Alternatively, mice infected with a TCT underproducing strain of B. pertussis exhibited pro-inflammatory responses. Addressing the nature of these responses requires access to large amounts of TCT and analogs containing chemical modifications. While there are established syntheses for TCT, they are difficult, restricted to labs with access to synthetic chemists, and can be arduous when introducing chemical modifications. This is due to the fragment possessing both a 1,6-anhMurNAc backbone and meso-diaminopimelic acid (mDap) residue in its peptide stem. However, bacteria are highly efficient at producing PG, and several species possess the recycling enzymes AmgK/MurU which can allow for the incorporation of modified MurNAc sugars into their peptidoglycan. Conveniently, B. pertussis possesses these enzymes, allowing for incorporation of 2-N-MurNAc modified probes into PG biosynthesis. Herein we report the use of a B. pertussis ΔAmpG mutant to ferment complex 1,6-anhydro and mDap containing PG fragments. This approach enables the creation of bioorthogonally labeled B. pertussis media and access to a library of chemical diverse TCT analogs. Ultimately, these new chemical tools can be used to further explore B. pertussis derived PG fragments and their role in the innate immune system.

(P-066) Enzymatic Differentiation and Diversification of Multi-antennary N-Glycans Enabled by Naturally Occurring Moieties on Glycans as Novel Protecting Groups

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Glycoproteins often exist in nature as heterogeneous mixtures, making the synthesis of homogeneous glycoforms essential for studying the role of glycosylation. Although the various chemical and enzymatic methods have been developed for the synthesis of N-glycans, these methods require extensive manipulations of organic protecting groups that are incompatible with water, making access to highly diverse N-glycans remains a challenge. Herein, we report a concise strategy for the synthesis of multi-antennary N-glycans in water, with sulfate and phosphate groups serving as orthogonal protecting groups. These charged groups are stable at physiological pH, reliably introduced in regioselective manner, and selectively removable by corresponding hydrolases to expose specific hydroxyl groups for enzymatic elaboration. Toward the chemical synthesis of the key octasaccharides for enzymatic elaboration, we solved a long-standing challenge in carbohydrate synthesis by achieving global demasking of multiple N-trichloroacetylamides (N-TCAs) under simple and mild hydrogenolytic conditions to unleash N-acetylamides (N-Ac), along with following debenzylation and azido reduction, overcoming the historically incomplete dechlorination.For antennae diversification, sulfate- and phosphate directed site-selective fucosylation on type II N-acetyllactosamine (N-LacNAc) by human fucosyltransferases (FuT-5, FuT-6, and FuT-9) based on their substrate preferences. Moreover, the 3O-sulfated galactose on bi-antennary N-glycans was tolerated by GnT-IV and GnT-V to afford tri-antennary, GlcNAc bifurcated products. Thus, this robust platform generated dozens of complex N-glycans with asymmetric antenna decorations. Our strategy repurposes sulfate and phosphate groups, twohydroxyl-decorating motifs common to biologists yet overlooked by synthetic chemists, into synthetically useful tools, leading to a general strategy for access complex carbohydrates and expansion of synthetic logic in organic synthesis.

(P-067) Chemoenzymatic Synthesis of Human Mucin-Mimicking Glycopolypeptides

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Mucus is a natural hydrogel secreted by epithelial cells. Mucus covers over 400 m2 of epithelial surfaces in the human body. It plays a critical role in biological functions, including protection against pathogens, lubrication and hydration of the epithelial surface, selective passage of nutrients and desirable molecules, cellular signaling, and microbiome support. Mucins are the major structural components of mucus, characterized by rigid amino acid backbones and heavy O-glycosylation. Over 20 MUC genes and numerous enzymes are involved in mucin secretion and glycosylation. Altogether, they contribute to structural complexity and heterogeneity across and within species and tissue types, making mucins challenging to study.Human mucins are also difficult to collect in bulk for biomedical studies. Animal mucins or traditional synthetic polymers are widely used as alternatives. However, these are not tunable for glycans, lack human-specific glycosylation patterns, or lack polypeptide backbones and native protein conformations. Mucin O-glycans frequently undergo physicochemically critical terminal modifications, such as sialylation, fucosylation, and sulfation that vary with diseases and that could affect barrier structure and function.This presentation will describe advances in synthesis chemically defined mucin mimetics and structure-function discovery of mucin physical and biological properties. We use glycosylated amino acid NCA polymerization to prepare high molecular weight polypeptides with tunable glycan identity and density. The generated synthetic mucin-mimicking polypeptides have revealed that glycosylation affects protein structure and biological function, and we expect applications in drug delivery systems, novel disease models, or as potential anti-infectives.

(P-068) Synthesis of Sialy-Tn- Modified MUC1 Peptides for Lectin Studies

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Aberrant cell-surface glycosylation is a hallmark of many cancers and contributes to tumor progression, metastasis, and immune evasion through interactions with carbohydrate-binding proteins (lectins). Tumor-associated carbohydrate antigens (TACAs), including the Thomsen Nouveau (Tn) antigen and Thomsen–Friedenreich (TF) antigen, and their sialylated analogs, are commonly displayed on the tumor-associated MUC1 (TA MUC1), where they mediate recognition by lectins. Our research aims to synthesize glycosylated amino acid building blocks carrying sialylated Tn and TF antigens for incorporation into solid-phase peptide synthesis, enabling the generation of defined glycopeptide models of TA MUC1. A novel, fully orthogonal strategy using phenylthiosialoside as the sialyl donor and the C6-OH Thr Tn building block as the glycosyl acceptor afforded the sialyl Tn-Thr building block in 70% yield under N-iodosuccinimide/trimethylsilyl trifluoromethanesulfonate (NIS/TMSOTf) activation condition. In contrast, applying the same conditions to the Ser acceptor produced the sialyl-Tn Ser building block in only 30% yield, underscoring the challenge of forming α-sialoside linkages due to competing nucleophilic substitution and elimination pathways. To provide mechanistic background for glycosylation efficiency, and the structural and conformational differences between the Thr and Ser acceptors we performed computational chemistry studies on the underlying reaction mechanisms. We further evaluated sialic acid donors bearing a 2,2,2 trichloroethoxycarbonyl (Troc) group at the C-5 amino position, as well as donors derived from the 5N,4O-oxazolidinone, for their effectiveness as sialyl donors in forming the sialyl Tn Ser building block. Replacement of the C-5 N-Acetyl with N-Troc resulted in no detectable glycosylation product while the cyclic oxazolidinone improved the yield to 50%. Concurrently, a new total synthesis approach towards the sialyl-TF (sTF) antigen is being developed. Key protected building blocks incorporating a pentafluoro phenyl (Pfp) ester at the C-terminal carboxyl group of the Thr residue have been synthesized and characterized, providing an activated carboxyl functionality suitable for subsequent solid phase peptide synthesis (SPPS). Together, these studies provide new insight into the synthesis of sialylated TACA building blocks and expand synthetic strategies towards sTn and sTF containing glycopeptides for the study of TA-MUC1.

(P-069) Synthesis of peptidoglycan fragments containing a 6-O-acetylation modification for therapeutic antibody production

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The rise of treatment-resistant pathogens is a growing threat to public health, exacerbating the need for the development of new therapeutics. The Gram-positive bacteria Staphylococcus aureus (S. aureus) has evolved to evade the host immune system and resist first and second-line antibiotics (methicillin/vancomycin). Bacteria accomplish this via modification of the peptidoglycan (PG) in their cell wall, including 6-O-acetylation of the N-acetyl-muramic acid (NAM) unit to evade glycosidic cleavage via the human protein lysozyme. Our lab is synthesizing PG fragments decorated with clickable alkyne handles, which can be conjugated to virus-like nanoparticles (VLPs) to produce glycoconjugates. These adjuvants have the capacity to activate a B-cell-dependent immune response, leading to the generation of high-affinity monoclonal antibodies (mAbs). Preliminary data demonstrates that this glycoconjugate pipeline can produce antibodies with therapeutic potential and selective glycan binding. Here, we demonstrate the synthesis of PG fragments, both mono-and disaccharides, including the 6-O-acetylation of the NAM fragment. With these fragments in hand, glycoconjugates can be produced to generate highly selective and specific therapeutic mAbs to treat antimicrobial-resistant pathogens.

(P-070) A catch and release strategy for cell surface glycoproteomics with intact glycosite information

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Glycosylation patterns, specifically on the cell surface, play a fundamental role in numerous biological processes, and aberrant regulation of cell surface glycosylation is linked to myriad diseases. Despite this importance, methods to capturing the cell surface glycoproteome remain limited by their reliance on protein-centric approaches where combinatorial glycan-amino acid epitopes are compromised as part of the workflow to enrich and elute cell surface proteins. This strategy not only limits cell surface glycoproteome characterization to a subset of glycoproteins but also perpetuates an incomplete framework for studying cell surface glycobiology. Here, we investigate an alternative labelling strategy to characterize intact glycopeptides enriched directly from the cell surface. A bifunctional labelling reagent was designed and synthesized in-house, incorporating two key functionalities: (1) a glycan-reactive handle for selective labelling and (2) an immobilized metal affinity chromatography (IMAC)-amenable group for downstream enrichment. We evaluated the labelling characteristics and stability of the probe under chemical and enzymatic conditions relevant to glycoproteomic sample preparation. Mass spectrometric analysis of labelled glycopeptides was used to confirm incorporation of the tag and assess its stability throughout the analytical workflow. We further investigated intelligent data acquisition mass spectrometry strategies designed to selectively identify tagged glycopeptide precursors for fragmentation. By capturing intact glycopeptides, this approach aims to preserve glycan–peptide connectivity and enable more detailed characterization of site-specific glycosylation directly at the cell surface.

(P-071) Covalent capture of transient protein-ligand interactions with squaramide ester probes

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Microbial lectins mediate host colonization, biofilm formation, and immune recognition, yet the vast majority of microbial carbohydrate-binding proteins remain uncharacterized (1) (2). Glycan arrays enable specificity profiling of known lectins, but no comparable method exists for the reverse problem: identifying unknown protein binders of a defined glycan. To address this gap, we have investigated monosquaramide ester electrophiles for covalent capture of binding events between proteins and low-affinity ligands, such as monovalent lectin-glycan interactions. These reactive handles are synthetically compatible with glycan moieties, making them well-suited for sugar-directed labeling. We identified structural features that modulate monosquaramide ester probe kinetics and demonstrated that these electrophiles react with greater selectivity near the probe binding site than other common reactive handles. Preliminary results leveraging this technology to label glycan-binding proteins on the surface of live microbial cells will be described.

(P-072) Preparative Separation of α2,3- and α2,6-Sialylated N-Glycans via Linkage-Specific Charge Modulation

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Sialylated glycans are essential biomolecules in glycoscience, with linkage variations—particularly α2,3- and α2,6-linked sialic acids—playing critical roles in glycan–protein interactions and disease processes. While analytical techniques can distinguish these linkages through differential chemical reactivity, such as lactone or ester formation that induces characteristic mass shifts, preparative separation is challenging due to similar chromatographic behavior. We report a charge-based strategy for efficient preparative separation of α2,3- and α2,6-sialylated glycans using weak anion exchange (WAX) chromatography. This approach exploits lactone and ethyl ester formation and their differential hydrolysis rates to modulate net charge, enabling milligram-scale isolation of natural glycans from diverse sources. The purified glycans were immobilized on mi-croarrays to investigate glycan-binding specificities of plant lectins and Siglecs, providing a robust platform for functional glycomics. investigators through comprehensive experimental consultation, technical training, and hands-on operational assistance across all platforms.

TOPIC 8: Glycoengineering and Translational Therapeutics

(PT-012) Programmable Glycocalyx Engineering Reveals Glycodomain Control of MUC1 Signaling and Dimerization

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Mucins are critical structural and signaling components of the glycocalyx, yet their large size and heterogeneous glycosylation have complicated efforts to define how the extracellular glycodomain regulates intracellular signaling. Mucin 1 (MUC1) is a transmembrane mucin comprising a heavily glycosylated extracellular domain (ED) and a signaling-capable cytoplasmic domain (CD) containing multiple phosphorylation sites. Existing approaches to study MUC1 have largely examined the ED or the CD separately, limiting direct investigation of how the ED structure influences MUC1 signaling. Here, we developed a programmable "mucin chimera" that combines synthetic, tunable glycodomains with MUC1's native signaling capabilities. Mucin chimeras are assembled with sortase-mediated ligation on living cells – synthetic glycodomains (synGDs) are covalently coupled to glycodomain-deleted MUC1, which retains its CD. These synGDs were synthesized via N-carboxyanhydride (NCA) polymerization and can therefore be freely adjusted in length, glycan density, glycan identity, and backbone composition and can exceed 300 amino acid residues. Chimeras displaying diverse glycans, including Tn antigen and sialylated/fucosylated structures, were successfully assembled on the cell surface. The resulting mucin chimeras retain native signaling capability and respond to extracellular stimulation through the MAPK/ERK pathway. Unexpectedly, glycosylated synGDs promote MUC1 dimerization, whereas their non-glycosylated counterparts do not. This glycosylation-dependent dimer is resistant to multiple reducing agents. Glycosylated synGDs also enhance MUC1 phosphorylation. In contrast, both glycosylated and non-glycosylated extracellular domains enhance

downstream ERK1/2 activation relative to glycodomain-deleted MUC1. Together, these findings establish mucin glycodomains as active regulators of transmembrane signaling. By integrating the chemical control of synthetic glycopolypeptides with native MUC1 signaling domains, this strategy enables systematic investigation of how glycan identity, density, and glycodomain stiffness shape signaling at the cell surface. The mucin chimera is a versatile addition in the glycobiology toolbox to further understanding of glycocalyx structure–function relationships.

(P-073) Expanding the Substrate Scope of Galactose Oxidase for Chemoenzymatic Glycoconjugate Vaccine Synthesis

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Glycoconjugate vaccines are among the most effective strategies for generating protective immunity against bacterial pathogens whose surfaces are decorated with complex polysaccharides. These vaccines convert polysaccharides, which are typically T-cell-independent antigens, into T-cell-dependent antigens by covalently linking them to immunogenic carrier proteins, eliciting adaptive immune responses capable of generating long-term, protective immunity. Despite their success, the chemical methods used to produce glycoconjugate vaccines remain largely empirical. Polysaccharide-protein conjugation frequently relies on non-specific chemical oxidation, a poorly controlled process that can alter polysaccharide structure and destroy immunologically important functional groups, potentially compromising antigenicity and vaccine efficacy. An alternative approach is chemoenzymatic oxidation using galactose oxidase (GOase), which can selectively oxidize primary alcohols within carbohydrate substrates and thereby introduce reactive handles for controlled conjugation. We have demonstrated that selective enzymatic oxidation of Streptococcus pneumoniae serotype 14 capsular polysaccharides (CPS) with GOase improves the chemical and immunological properties of the resulting glycoconjugate vaccine, producing enhanced antibody responses compared with traditionally prepared formulations. However, the substrate specificity of GOase restricts the types of polysaccharides that can be functionalized using this approach. To expand the applicability of enzymatic oxidation for glycoconjugate vaccine synthesis, we are engineering GOase variants with altered and broadened carbohydrate specificity. Directed evolution, coupled with high-throughput fluorescence-activated cell sorting (FACS)-based screening and combinatorial active-site saturation mutagenesis, enables the screening of large enzyme libraries for substrate-dependent oxidation and the identification of variants with enhanced activity toward carbohydrate structures that are not substrates of wild type GOase. Pneumococcal CPSs, including serotype 3 CPS containing terminal glucose residues, provide important targets for developing and evaluating these new catalytic activities. Generating GOase variants with expanded substrate scope will provide a versatile enzymatic toolkit for selective polysaccharide functionalization and controlled glycoconjugate synthesis. By replacing non-specific chemical oxidation with programmable enzymatic modification, this approach could preserve antigen structure, improve conjugation chemistry, and enable the rational design of more structurally defined next-generation glycoconjugate vaccines.

(P-074) Programmable biosynthesis of human mucin-type O-glycans in a one-pot cell-free platform

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Synthetic glycobiology has the potential to transform the production of glycoproteins with defined glycan structures for applications in cancer diagnostics, therapeutics, vaccine development, and biologic manufacturing. Among the diverse classes of protein glycans, mucin-type O-glycans remain particularly challenging to engineer as their biosynthesis requires the sequential action of multiple glycosyltransferases (GTs) whose expression varies across tissues and cell types, resulting in highly heterogeneous glycoforms. Consequently, the production of structurally defined O-glycoproteins remains largely dependent on mammalian cell expression systems, where membrane-bound Golgi-resident GTs are difficult to manipulate and glycan composition is challenging to control. Scalable biosynthetic platforms capable of generating programmable O-glycoforms are therefore needed to enable mechanistic studies of glycan function and accelerate the development of designer glycoproteins for biomedical applications.To address this challenge, we developed a one-pot cell-free glycoengineering platform using Escherichia coli-derived lysates enriched with defined O-glycosylation enzymes for the programmable assembly of human mucin-type O-glycans. Central to this platform is SIMPLEx, an

inhouse protein engineering strategy that enables soluble expression of membrane-associated GTs by combining an N-terminal maltose-binding protein (MBP) translational decoy to prevent membrane insertion with a C-terminal apolipoprotein A1 (ApoA1) fusion that shields hydrophobic surfaces and promotes solubility in the bacterial cytoplasm. Using SIMPLEx, we generated soluble and catalytically active human GalNAcT2 and Drosophila melanogaster core 1 β1,3-galactosyltransferase (dmC1GalT1), which were incorporated into enzyme-enriched lysates for one-pot glycosylation reactions. As a proof-of-concept substrate, we selected and designed a recombinant MUC1 because mucins are among the most extensively O-glycosylated proteins in nature, providing a stringent model for evaluating sequential O-glycan assembly. Sequential installation of the Tn and core 1 (T) antigens on MUC1 was confirmed by Vicia Villosa agglutinin (VVA) and Peanut Agglutinin (PNA) lectin blotting, respectively. Collectively, these results establish a modular cell-free platform for programmable O-glycan biosynthesis and provide a versatile framework for generating designer O-glycoproteins with defined glycoforms for mechanistic glycobiology, therapeutic protein engineering, vaccine development, and cancer research.

(P-075) Glycopolymers enrich less immunogenic nanoparticle protein corona and improve tumor delivery compared to Poly(ethylene glycol)

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Though PEGylated nanoparticles (NPs) are common, it is increasingly appreciated that PEG causes immunogenic reactions and is susceptible to rapid clearance via the binding of anti-PEG antibodies. As an alternative to PEGylation, we engineered NPs composed of a diblock copolymer, poly(2-(diisopropylamino)ethyl methacrylate)-b-poly(methacrylamidoglucopyranose) [PDPA-b-PMAG]. We compared the physicochemical characteristics, biocompatibility, cell uptake, protein corona composition, and in vivo delivery of PMAG and PEG NPs. We found that PMAG and PEG NPs could be produced with similar physicochemical characteristics (e.g., size, surface charge, and morphology) and cellular biocompatibility. However, these NPs exhibited vastly different biological interactions which were dependent upon the presence of the NP protein corona. Compared to PEG NPs, PMAG NPs exhibited roughly 5-fold higher uptake in MDA-MB-231 breast cancer cells in culture. PMAG NPs also exhibited significantly less uptake than PEG NPs in RAW 264.7 macrophages. Pretreatment of the NPs with human serum further increased the uptake of PEG NPs in RAW 264.7 macrophages while PMAG NP uptake in the macrophages reduced. Protein corona analysis revealed that PMAG NPs adsorb less immunogenic proteins than PEG NPs. For instance, the abundance of IgG-class and complement proteins was reduced ~5-fold and ~10-fold, respectively, on the surface of PMAG NPs compared to PEG NPs. We tested the functional significance of these observations by comparing the pharmacokinetics and tumor delivery of PMAG and PEG NPs in a murine model of triple negative breast cancer (TNBC). In this model, PMAG NPs had 2.5-fold higher circulation time and 2-fold higher tumor delivery than PEG NPs. Additionally, PMAG NPs achieved a 3:1 tumor:liver delivery ratio, ~3-fold higher than that of PEG NPs. Thus, glycopolymer-based PMAG NPs represent a promising alternative to PEG NPs, reducing immunogenicity and improving tumor delivery in a model of TNBC.

(P-076) Metabolic glycoengineering for producing site-specific antibody-drug conjugates

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Metabolic glycoengineering (MGE) approaches aim to exploit the natural biosynthetic pathway to modulate the glycosylation of cellular glycoconjugates, including the incorporation of unnatural and functionalized sugars. While metabolic glycoengineering strategies have been explored for the labeling of cell surface glycoconjugates, the incorporation of derivatized monosaccharides into specific extracellular proteins has been more overlooked. Ample previous studies have shown that the N297 Fc glycan of immunoglobulins (IgG) plays essential roles in modulating the immune response, stability and pharmacokinetics of antibodies. Specifically, it has been reported that Fc sialylation is responsible for the anti-inflammatory activities of intravenous immunoglobulins and sialylated Fcs. However, natural and recombinant antibodies typically have less than 10% sialylation. Here, we report a metabolic glycoengineering strategy wherein an F241A point mutation combined with co-expression of the antibody with a human β1,4-galactosyltransferase (B4GalT1) and/or the α2,6-sialyltransferase (ST6Gal1) greatly enhances sialylation of the

N297 Fc glycan in HEK293 cell lines. Further, supplying cells with functionalized N-acetylmannosamine analogs, i.e. Ac4ManNAz and Ac4ManN(F-Ac), as the sialic acid precursors is a highly effective strategy to achieve significant levels of incorporation of non-natural sugar analogs into the Fc glycan. This strategy allows for the remarkable incorporation of selectively modified ManNAc analogs for the labeling and functionalization of sialylated antibodies. Using this approach to produce site-specifically labelled antibodies enables the manipulation and control of antibody Fc glycoforms and also provides a novel cell-based method for constructing site-specific antibody-drug conjugates and antibody-ligand conjugates at the native IgG Fc glycosylation site.

TOPIC 9: Glycoconjugate Biosynthesis and Degradation

(KEY9-001) Diversity and Evolution of the Vertebrate Sialyltransferase Repertoire

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Sialic acids (SA) are commonly found on cell surface glycoproteins and glycolipids. Biosynthesis of sialylated molecules is an essential pathway that controls cellular functions like embryonic development [1]. It involves biosynthetic enzymes known as sialyltransferases (ST), that catalyze the stereo- and regio-specific transfer of SA from activated sugar donors (CMP-SA) to a variety of acceptor substrates [2]. We use bioinformatics to identify animal sialyltransferase genes of the CAZy GT#29 and predict their function. Four ST families (ST3GAL, ST6GAL, ST6GALNAC and ST8SIA) are identified in Metazoa [3]. Phylogenomic and protein sequence analyses shed light into the origin ST and SA pathway in Eukaryota, Bacteria and Archaea: the major actors of the SA pathway are already present in the Last Common Ancestor of Eukaryotes (LECA) [4]. Comparative genomics analyses highlight the diversity and heterogeneous distribution of the ST genes among Metazoa. Sequence-based analyses reveal fundamental mechanisms governing sialylation reaction, and unveiled potential changes of function after gene or genome duplications as exemplified by the ST8SIA family [5]. To characterize human and novel vertebrate ST activities, we developed a glyco-tool box with engineered recombinant enzymes and chemo-enzymatically synthetized donor substrates (both natural and functionalized) and we set up cell-free ST assays on microplates (MPSA) [6-7]. Computational approaches and molecular dynamic simulations were used to investigate the structural and biochemical determinants of the donor substrate specificity in the polysialyltransferase ST8Sia IV and determine their evolution in vertebrates [8].Collectively, our data underscore molecular and functional evolution of vertebrate ST that account for the wide diversity of vertebrate sialoglycoconjugates. They provide useful strategies and information revealing fundamental biology of SA, and identifying therapeutic targets.References: 1. Douchez P, Fliniaux I, Takeda-Uchimura Y, Marin M, Martoriati A, Harduin-Lepers A, Cailliau K. Cells Dev. 2026, 185:204057.2. Harduin-Lepers A. Glycoconj. J. 2023, 40, 473–492.3. Harduin-Lepers A, Mollicone R, Delannoy P and Oriol R. Glycobiology 2005, 15, 805-8174. Petit D., Teppa E., Cenci U., Ball S., Harduin-Lepers A. Scientific reports 2018, 8(1):29465. Venuto M.T., Decloquement M., Ribera M. J., Noel M., Rebl A., Cogez V., Petit D., Galuska S.P., Harduin-Lepers A. IJMS 2020, 21(2), 5136. Decloquement M, Venuto MT, Cogez V, Steinmetz A., Schulz C, Lion C, Noel M, Rigolot V, Teppa RE, Biot C, Rebl A, Galuska SP and Harduin-Lepers A. Scientific reports 2023, 13, 156107. Filipe KC, Dangudubiyyam S, Lion C, Decloquement M, Elin Teppa R, Biot C, Harduin-Lepers A. Chembiochem. 2025, 26(5):e202400539.8. Teppa RE, Galuska SP, Harduin-Lepers A. BBA GS 2024, 1868(8):130647.

(KEY9-002) Lectin domain-mediated regulation of glycosyltransferases for N-glycan branching and LacdiNAc biosynthesis

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N-glycans exhibit enormous structural variation, which critically regulates glycoprotein function. Such structural variations are produced by many glycosyltransferases that generate GlcNAc branches and terminal modifications, such as sialylation and fucosylation. We have been working on the activity regulation of these glycosyltransferases to understand how various N-glycans are shaped in mammalian cells.Among the glycosyltransferases involved in N-glycan branching, MGAT4A and B (GnT-IVa and -IVb) synthesize the β1,4-GlcNAc branch, and MGAT4A is involved in type 2 diabetes. We discovered that these enzymes have a unique C-terminal lectin domain and solved its crystal structure and glycan-binding specificity (Nagae et al., Commun., Biol., 2022).

Furthermore, we recently found the unique glycosylation mechanism of these enzymes, in which the lectin domain recognizes glycoprotein substrates for efficient branching. Furthermore, this process is self-regulated by its own N-glycan attached to the lectin domain (Osada et al., iScience, 2024). This indicates that MGAT4 activity is uniquely self-regulated by its own glycan.We also identified a unique lectin domain (PA14 domain) in LacdiNAc synthases, B4GALNT3 and B4GALNT4. LacdiNAc (GalNAcβ1-4GlcNAc) was reported to be involved in the clearance of glycoproteins from circulation and in maintenance of cell stemness. However, the mechanism by which B4GALNTs activity is regulated in cells and how LDN plays different roles from the major sub-terminal structure LacNAc remain largely unknown.We recently showed that LDN suppresses the actions of many glycosyltransferases for terminal modifications, including sialylation, fucosylation, and human natural killer-1 (HNK-1) biosynthesis (Tokoro et al., J. Biol. Chem., 2024). Moreover, we recently found that B4GALNT3 suppresses N-glycan branching and vice versa, indicating that LDN formation and GlcNAc branching are mutually suppressive processes. Because LDN biosynthesis suppresses both branching and terminal modifications, N-glycan structures become simplified by B4GALNT3 overexpression. We also found that B4GALNT3 and 4 have a unique lectin domain that specifically binds to sulfated glycans (Tokoro et al., J. Biol. Chem., 2026). The addition of small glycan ligands inhibited the activity of the catalytic domain, suggesting that LDN biosynthesis is regulated by sulfated glycans mediated by the lectin domain. Taken together, LDN has a significant impact on N-glycan maturation in a completely different way from LacNAc, and B4GALNT3 and 4 have unique structures and regulatory mechanisms.

(PT-013) Human milk oligosaccharide degradation by microbial sialidases in the breastfed infant gut microbiome

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Breastmilk and formula contain markedly different compositions of human milk oligosaccharides (HMOs), specifically those with sialic acid. HMO composition is a key factor influencing the gut microbiome composition of breastfed and formula-fed infants, with breastfed infants having less diverse, but more beneficial gut microbes. The reason why beneficial bacterial thrive on the composition of HMOs in breastmilk is not fully understood. Interestingly, sialylated HMOs remain undigested by human enzymes in the gastrointestinal tract and instead are metabolized by microbial enzymes called sialidases. Therefore, we suggest microbial sialidases as a molecular mechanism that beneficial bacteria employ to more efficiently utilize sialylated HMOs found in breastmilk. Using a structure-based rubric, we identified microbial sialidases in both adult and infant metagenomic datasets. Eleven diverse sialidases were selected for biochemical characterization with individual sialylated HMOs, as well as a biologically relevant mixture isolated from donor breastmilk. Further structural characterization of sialidases was conducted to identify critical amino acid residues that contribute to sialylated HMO turnover. Finally, early-life breastfed and formula-fed microbiome metagenomic data was examined for sialidase-producing species abundances and unique species counts, revealing increased beneficial sialidase-producing species in breastfed infants, especially at 1 month after birth. Thus, sialidases are an important mechanism used by beneficial bacteria to thrive on sialylated HMOs in breastfed infant gut microbiomes. Therefore, sialylated HMOs, such as 3'sialyllactose, could potentially be supplemented in infant formulas to support healthy microbiome development and overall infant health.

(P-077) The 40th anniversary of deaminoneuraminic acid (Kdn): Its unique properties and biological significance

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Sialic acid (Sia) is a group of nine-carbon acidic sugars and divided into three molecular species by a substituent group at C5 position: N-acetylneuraminic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc) and deaminoneuraminic acid (Kdn). Kdn was discovered in 1986 in the cortical vesicular polysialoglycoproteins of rainbow trout eggs (Nadano D et al., 1986). A lot of studies on the occurrence, structure, metabolism, and function have since been carried out. In 1999, we were the first to elusidate the metabolic

pathway of Kdn using rainbow trout gametic cells (Angata T et al., 1999). For the biosynthesis, Kdn monosaccharide is synthesized from mannose (Man) or Man 6-phosphate (Man-6-P) as precursor sugars. Man-6-P is condensed with phosphoenolpyruvate (PEP) to form Kdn-9-P, followed by dephosphorylation to give free Kdn. Kdn is then activated to CMP-Kdn with CTP. Kdn residues are synthesized by sialyltransferases using CMP-Kdn as a donor substrate. For the degradation, Kdn-glycosides are hydrolyzed by sialidases into free Kdn, which is further degraded into Man and pyruvate by Kdn-aldolases. Interestingly, the enzymes involved in the biosynthesis of Kdn and its glycosides are essentially the same as those involved in the metabolism of Neu5Ac, except the absence of UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE) involvement. However, depending on the substrate specificity, which often differs among species, the fates of Kdn and Neu5Ac differ in terms of structure and function. For example, mammalian CMP-sialic acid synthetases (CSS or CMAS) can hardly activate Kdn to CMP-Kdn. Therefore, in mammals Kdn residues are not expressed, while free Kdn is increased if Man and Man-6-P are provided. It can be recognized that the synthesis of Kdn is a detoxification reaction of Man and Man-6-P, which are sometimes hazardous to cell viability. This is a unique feature of Kdn metabolism in mammals.One of the prominent features is that Kdn-residues are resistant to most viral, prokaryotic and eukaryotic sialidases. However, we previously discovered KDNase, a unique sialidase that can hydrolyze Kdn residues, but not Neu5Ac or Neu5Gc, in a Sphingobacterium sp. We recently analyzed the whole genome of this Sphingobacterium species, and found the presence of Kdn-aldolase gene, which exclusively degraded free Kdn, but not Neu5Ac or Neu5Gc (Nakagawa T et al., 2023). Interestingly, the Kdn-aldolase gene is situated adjacent to the KDNase gene, forming a Kdn-specific catabolic operon. Furthermore, the Kdn-specific operon was found to be independent of the Neu5Ac catabolic operon, indicating the importance of Kdn-specific metabolism in this organism.

TOPIC 10: Host–Pathogen Interactions, Microbial and Infectious Diseases

(KEY10-001) Intracellular galectins in host-microbe interaction through binding cytosolically exposed host or microbial glycans

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Galectins are beta-galactoside-binding lectins containing one or two conserved carbohydrate-recognition domains (CRD). They do not contain a classical leader sequence and are found mostly inside the cells, although they can be released by cells. Both extracellular and intracellular functions have been demonstrated for these proteins, with the latter having largely been linked to the proteins interacting with other intracellular signaling molecules in a carbohydrate-independent fashion. An intriguing recent development is that intracellular galectins can act as sensors of damaged organelles by binding to host glycans that become exposed to the cytosol. Galectin-8 was shown to bind Salmonella-damaged vacuoles and target them for autophagy, resulting in degradation of bacteria. We found galectin-3 accumulated around phagosomes containing Listeria monocytogenes, through binding to host N-glycans on the membrane of damaged phagosomes, and subsequently suppressed autophagic activation induced by the bacterial infection. We also found appearance of galectin-3 and galectin-8 aggregates around damaged lysosomes in the gastric epithelial line AGS cocultured with Helicobacter pylori; this occurred in a host O-glycan-dependent manner. These were followed by various cellular responses, including apoptosis and autophagy. Intracellular galectins can also recognize glycosylated bacterial components released into the host cytosol or glycans on the surface of intracellular bacteria. By binding to cytosolic lipopolysaccharides (LPS), intracellular galectin-3 augments noncanonical inflammasomal activation mediated by LPS that involves caspase oligomerization and activation. Galectin-4 can recognize glycans on invading intracellular Salmonella, leading to their aggregation, as well as induction of inflammasome activation. Thus, the galectin family members have emerged as regulators of intracellular innate immunity that function through recognition of host and microbial glycans.

(KEY10-002) Engineering Cell-Based Platforms to Decode Glycan Recognition and Host-Microbe Interactions

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Mucins and mucin-like proteins contain heavily O-glycosylated domains that have long remained inaccessible to detailed structural and functional studies due to their dense O-glycan decoration. We sought to capture the biological information encoded within these O-glycodomains and enable systematic dissection of the molecular cues governing glycan-mediated interactions. To this end, we developed a cell-based glycoengineering platform to display and produce O-glycodomain reporters with defined O-glycans in genetically engineered mammalian cells. Surface display of these O-glycodomain reporters serves as a versatile cell-based glycan array with protein context for probing and dissecting interactions on cells, while the same engineered cells enable scalable production of secreted reporters for assays with isolated reporters. Expanding this mucin reporter concept with Glycocarriers containing short repeated glycosylation motifs without resemblance to human proteins provides access to homogeneous glycans without context as released oligosaccharides, glycopeptides, and multivalent glycodomains. We have illustrated the utility of this platform to define the binding specificities of diverse glycan-binding proteins, including antibodies, innate lectins, microbial adhesins, and viruses, and to uncover previously unrecognized mechanisms of contextual glycan recognition. I will illustrate this with the first mucin-binding module X409 that recognizes a clustered saccharide patch formed by adjacent O-glycans, and with the novel microbial di-glutamate family of mucinases that similarly recognize an extended cluster of truncated O-glycans found on select mucins for cleaving mucins following trimming of their O-glycans by microbial grazing. These studies begin to reveal how gut microbes recognize, bind, and degrade mucins during mucus turnover and microbial foraging. The versatile cell-based platform for sustainable production of defined glycoconjugates and systematic dissection of glycan-mediated interactions is continuously being expanded, and new applications are being developed to address specific challenges.References1. Narimatsu Yet.al., A Family of Di-Glutamate Mucin Degrading Enzymes that Bridges Glycan Hydrolases and Peptidases. (2024) Nat Catalysis.2. Jaroentomeechai T et.al., Mammalian cell-based production of glycans, glycopeptides and glycomodules. (2024) Nat Commun 1;15(1):9668.3. Jaroentomeechai T et.al., Microbial binding module employs sophisticated clustered saccharide patches to selectively adhere to mucins. (2025) Nat Commun. 16(1):9058.4. Narimatsu et.al., Discovery of a secreted Bacteroides fragilis mucinase that cleaves mucins with bis-T O-glycans through a carbohydrate binding module-dependent mechanism. (2026) Gut Microbes. 18(1):2644983.

(KEY10-003) Host glycan and mucin interactions with the vaginal microbiota associated with preterm birth

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The vaginal microbiome is a key determinant of reproductive health and plays an important role in modifying the risk of adverse pregnancy outcomes, including preterm birth and sepsis in the neonate. A microbiome dominated by Lactobacillus crispatus is associated with reproductive health and protection against preterm birth, whereas loss of beneficial lactobacilli, a hallmark of bacterial vaginosis, often leads to increased abundance of diverse and potentially pathogenic bacteria, and it is linked to inflammation and increased risk of preterm birth and neonatal sepsis. Glycans are central to host–microbe interactions providing attachment sites and nutrient sources for bacteria, while modulating mucosal immune response. However, how host glycans shape the vaginal microbiome remains poorly understood. Using glycan microarrays of sequenced defined glycans relevant to the genitourinary tract, we profiled glycan recognition by whole bacterial cells and revealed distinct glycan-binding patterns among vaginal bacteria species associated with different risk of preterm birth and neonatal sepsis (Tajadura-Ortega et al. Nat Comms 2025, PMID: 40467588). In this talk, I will present our recent work exploring host glycan interactions with vaginal bacteria associated with reproductive health and risk of preterm birth, including the ongoing development of patient-derived cervicovaginal mucin (CVM) microarrays as a physiologically relevant platform for investigating bacterial interactions with naturally occurring glycan repertoires at the individual level. By integrating sequence-defined glycan and CVM microarrays, we aim to understand how variation in the cervicovaginal mucosal glycome influences bacterial colonisation, microbiota composition and stability, and ultimately reproductive health.

(PT-014) Adiponectin recognizes bacterial lipopolysaccharide glycans to protect against enteric and systemic infection

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Host lectins recognize microbial glycans to preserve barrier integrity and prevent infection, yet the glycan-recognition repertoire at epithelial barriers remains poorly defined. Adiponectin is a highly abundant C1q-like protein best known as a metabolic hormone. Here, we identify adiponectin as a glycan-binding host-defense lectin active in the intestinal lumen and circulation. Microbial colonization induced ileal adiponectin expression, and Paneth cells secreted adiponectin into the intestinal lumen. Enteric challenge with Citrobacter rodentium revealed that whole-body and intestinal epithelial cell-specific adiponectin deficiency increased bacterial association with the epithelial surface and penetration into the inner mucus layer. In contrast, adiponectin loss did not alter Salmonella Typhimurium burdens, indicating pathogen-selective protection. These contrasting infection phenotypes suggested that adiponectin differentially recognized the two bacteria. Recombinant adiponectin bound C. rodentium more efficiently than S. Typhimurium, implicating bacterial surface features in pathogen selectivity. Adiponectin bound to lipopolysaccharide with apparent nanomolar affinity, preferentially recognizing its polysaccharide portion rather than lipid A or the inner core. Further, glycan array analyses revealed preferential recognition of O-antigens enriched in acidic and N-acetylated sugar moieties. In a systemic Pseudomonas aeruginosa infection model, adiponectin deficiency increased susceptibility, whereas adiponectin supplementation improved infection outcomes. Together, these findings identify adiponectin as a pattern-recognition lectin that links bacterial O-antigen glycan recognition to selective antibacterial defense at mucosal and systemic sites, broadening its biological role beyond metabolic regulation.

(P-078) HIV envelope glycopeptide specific human CD4$^+$ T cell cloning and TCR sequencing and characterization

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Acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus-1 (HIV-1) remains a major global health challenge, and an effective protective HIV vaccine has yet to be developed. Recent studies have demonstrated critical roles for CD4$^+$ helper T cells in driving antibody affinity maturation, subclass switching, and the development of broadly neutralizing antibodies (bNAbs). Therefore, understanding CD4$^+$ T-cell responses to the highly glycosylated HIV-1 envelope (Env) is important for developing effective vaccine strategies. The extensive glycan shield of Env not only influences antibody recognition but can also generate glycopeptide epitopes that are presented to CD4$^+$ T cells by MHC class II (MHCII). We previously demonstrated that gp120-derived glycopeptides can elicit glycan-dependent CD4$^+$ T-cell responses and promote potent Env-specific humoral immunity. Here, I have extended these studies to characterize human CD4$^+$ T-cell responses to HIV-1 Env glycopeptides. I have successfully enriched glycopeptide-responsive CD4$^+$ T cells and established approaches to characterize their functional and T-cell receptor (TCR) profiles. Preliminary TCR sequencing analyses suggest distinct clonotypic patterns among CD4$^+$ T-cell responses to glycosylated and corresponding non-glycosylated peptides, supporting the possibility that Env glycans contribute to T-cell specificity and TCR selection. Further characterization of these glycopeptide-specific CD4$^+$ T cells, or "Tcarbs," will determine their functional properties and potential role in providing T-cell help for Env-specific B-cell responses. Understanding how Env glycosylation shapes T-cell recognition may reveal a previously underappreciated link between glycan-dependent T-cell immunity and humoral responses and may inform the development of HIV vaccines designed to coordinate T-cell help with the induction of broadly neutralizing antibodies.

(P-079) Chemoenzymatic Synthesis of Bacterial UDP-Sugars to Investigate Host–Microbe Glycan Interactions

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Bacterial peptidoglycan (PG) biosynthesis generates a diverse array of nucleotide-linked sugar intermediates that serve as precursors to the bacterial cell wall and can influence host immune signaling. However, access to structurally defined bacterial UDP-sugar and peptidoglycan precursors remains challenging, limiting biochemical studies of their interactions with host glycosylation pathways and innate immune receptors. Here, we developed a chemoenzymatic platform for the synthesis and characterization of bacterial UDP-sugar and peptidoglycan precursors. By combining purified enzymes from bacterial PG recycling, we reconstructed selected biosynthetic steps in vitro to generate defined nucleotide-sugar products, including UDP-linked peptidoglycan intermediates. Enzymatic reactions were monitored using chromatographic and mass spectrometric approaches to confirm product formation and structural identity. This approach enabled the large-scale synthesis and purification of UDP-N-acetylmuramic acid (UDP-NAM), UDP-muramyl dipeptide (UDP-MDP), and azide-functionalized UDP-sugar derivatives.These structurally defined bacterial UDP-sugars were subsequently used to investigate their interactions with O-GlcNAc transferase (OGT), a central enzyme in mammalian glycosylation. Preliminary biophysical studies demonstrated ligand-dependent stabilization of OGT, with UDP-MDP producing measurable thermal stabilization, while binding studies supported differential interactions between OGT and bacterial UDP-sugar ligands. Molecular docking further identified a conserved OGT binding environment involving residues including Thr931, Lys908, and Asp935. Together, this work establishes a biochemical framework for defining the relationship between bacterial nucleotide-sugar metabolism and host glycosylation. Access to structurally defined bacterial UDP-sugar precursors provides valuable tools for investigating chemical communication at the host–microbe interface and may reveal previously unrecognized connections between bacterial cell-wall metabolism, mammalian glycosylation, and innate immune signaling.

(P-080) Identifying and characterizing conserved determinants of HIV Env glycoantigen recognition

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The HIV envelope (Env) glycoprotein is among the most densely N-glycosylated viral proteins, with its extensive and heterogeneous glycan shield playing critical roles in glycoprotein structure, immune evasion, and immune recognition. Despite substantial sequence diversity among HIV strains, conservation of N-glycosylation sites and glycan processing may generate overlapping motifs within the Env glycan landscape with functional consequences for both humoral and cellular immunity. In particular, N-glycans can form integral components of antibody epitopes, while glycosylated peptides generated during antigen processing may be presented by major histocompatibility complex II (MHCII) to CD4+ T cells. In previous work, our group identified a glycosylated HIV Env epitope capable of MHCII-dependent T cell recognition. Here, we seek to define conserved features of Env N-glycosylation across divergent HIV clades and investigate their relationships with immune recognition. We expressed native flexibly linked (NFL) Env trimers representing three major HIV clades, A (BG505), B (JR-FL), and C (16055), in Expi293 cells and characterized their N-glycan landscapes using mass spectrometry-based glycomic and site-specific glycopeptide analyses. Site-resolved N-glycosylation profiles are integrated into a cross-clade similarity network to identify conserved and divergent patterns of glycan processing across Env backgrounds. We are further investigating the consequences of Env N-glycosylation through complementary approaches examining cellular and humoral immune recognition. Candidate glycopeptides are evaluated for their interactions with MHCII, while a pseudovirus panel spanning diverse Env backgrounds is being developed to determine whether conserved N-glycosylation features are associated with monoclonal antibody neutralization sensitivity. Together, these approaches provide a framework for connecting site-specific N-glycan heterogeneity and conservation across the HIV Env glycan landscape with functional immune recognition. Defining conserved N-glycosylation features across genetically diverse Env proteins may reveal glycan-associated determinants of antigen presentation and antibody neutralization and provide insight into the role of the Env glycan shield in HIV immunit

(P-081) Context-Dependent Heparan Sulfate Recognition Regulates Arbovirus Dissemination

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Heparan sulfate (HS) is a ubiquitously expressed glycosaminoglycan that mediates protein-cell surface interactions and serves as a common attachment factor for many viruses. Many arthropod-borne viruses (arboviruses) acquire enhanced HS-binding properties as a cell culture adaptation, a trait that frequently correlates with viral attenuation and has been exploited in the development of live-attenuated vaccine candidates. In contrast, naturally circulating eastern equine encephalitis virus (EEEV) isolates exhibit strong HS-binding activity that is associated with high pathogenicity and pronounced neurovirulence, suggesting that EEEV exploits specific HS structures to promote infection rather than attenuation. As such, EEEV provides a compelling system to interrogate the molecular and contextual determinants of arbovirus–HS interactions in vivo. Through transient depletion of vascular HS in animal models and mutation of HS-binding sites on the viral particle, we find that EEEV engages HS in a highly host , tissue , and cell-specific manner. First, we observe HS-dependent clearance of EEEV viral particles from the blood circulation in a mammalian (mouse) model but not in an avian (chicken) model, paralleling the ecological roles of these hosts in EEEV transmission. Second, both wildtype EEEV viral particles and a mutant deficient in HS-binding traffick similarly to the draining lymph node early after footpad inoculation—suggesting that HS at the site of infection does not restrict initial dispersal of viral particles and reveals a previously unappreciated tissue specificity in EEEV-HS engagement. Third, EEEV interactions with vascular HS is associated with blood-filtering phagocytes. Depletion of this cell population is associated with enhanced viral dissemination at 1 day post inoculation, similar to a mutant virus deficient in HS-binding. On the viral particle itself, we have identified two basic patches on the viral E2 glycoprotein that are required for HS engagement in vivo. Interestingly, alanine substitution of any individual residue within either of the basic patches is sufficient to abrogate EEEV interactions with vascular HS. Together, these findings support a model in which viral dissemination is regulated by defined viral determinants engaging specific, context-dependent HS. Ongoing studies are focused on defining the HS structural properties that support these interactions and on developing complementary viral model systems to further elucidate how HS-virus interactions shape immune responses, viral pathogenesis, and ecological niches.

(P-082) Phosphatidylethanolamine is essential for asymmetric peptidoglycan synthesis and antibiotic resistance in Mycobacterium smegmatis

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Peptidoglycan is arguably the most important glycan polymer in bacterial cells. It maintains cell shape, withstands internal turgor pressure, and provides a scaffold for cell growth and division. In mycobacteria, which include Mycobacterium tuberculosis, the causative agent of tuberculosis, rod-shaped cells extend their peptidoglycan polymer asymmetrically from subpolar regions: the new pole (generated during prior division) grows less than the old pole. This asymmetric growth creates heterogeneity among offspring, and such diversity contributes to population resilience against antibiotic treatments. However, the underlying mechanisms remain largely unknown. Here, we show that a defect in phosphatidylethanolamine (PE) biosynthesis results in the loss of asymmetric subpolar peptidoglycan growth. We created a Mycobacterium smegmatis mutant with a deletion in the gene MSMEG_0861, which encodes phosphatidylserine decarboxylase (Psd), the enzyme predicted to mediate the final step of PE production. We found that psd deletion results in loss of PE, smaller colonies, altered growth in liquid culture, and increased antibiotic susceptibility. Furthermore, we found that loss of PE causes delocalization of an inner membrane domain (IMD)-associated protein from the polar region. Finally, we found that the Δpsd mutant synthesizes peptidoglycan more symmetrically from both poles than the wild type. Our results suggest that PE is required for asymmetric peptidoglycan synthesis in M. smegmatis, potentially through active enrichment of IMD-associated proteins to the subpolar region of the old pole.

(P-083) PsrP O-glycosylation is metabolically wired to central amino-sugar metabolism in Streptococcus pneumoniae

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Streptococcus pneumoniae (Spn) accounts for the majority of community-acquired pneumonia, kills roughly one million children under five annually, and is a World Health Organization priority pathogen with rising antibiotic resistance renewing prevention efforts. Spn is a Gram-positive, facultative anaerobic opportunistic pathogen that colonizes the nasopharynx and causes otitis media and invasive pneumococcal diseases including meningitis, community-acquired pneumonia, and sepsis. In serotype 4 strain TIGR4, the psrP-secY2A2 locus encodes PsrP, a 4,776-amino-acid serine-rich repeat (SRR) protein with two repeat regions, a short SRR1 and an extremely long SRR2 (~539 repeats), that are extensively O-glycosylated. The glycosyltransferases GtfA/B catalyze the first committed GlcNAc transfer onto serine residues and subsequently, additional glycosyltransferases extend these sites into a complex, mucin-like glycan decoration. PsrPs glycosylation is generally viewed as a structural requirement for display. However, glycosylation has consequences for the cell beyond adhesion is undiscovered. We hypothesize that the heavy amino-sugar demand of decorating hundreds of SRR2 repeats couples PsrP glycosylation to central metabolism, such that disrupting glycosylation is sensed and metabolically compensated. To test this, we transcriptionally profiled an isogenic TIGR4 panel, ΔpsrP (adhesin absent) and ΔgtfA/B (PsrP present but unglycosylated), against wild type by RNA-seq in planktonic culture. Both mutants did more than remodel the surface: they shifted expression across core metabolic pathways. Most strikingly, a carbohydrate ABC-importer solute-binding protein was upregulated only when glycosylation was blocked (ΔgtfA/B), not when the adhesin alone was deleted (ΔpsrP).Together these data support an expanded role of SRRP glycosylation as an amino-sugar linked metabolic process rather than a purely structural one. To connect this signature to the glycans itself we generated mutant strains to assess surface display across these backgrounds, and targeted amino-sugar metabolomics and promoter-binding assays are underway to define the regulators driving this compensatory program. Together these data support an expanded role for serine-rich repeat protein glycosylation as an amino-sugar-linked metabolic process rather than a purely structural one. Furthermore, PsrP glycans may be a promising vaccine target, as the immense size of the protein allows these epitopes to extend past the capsular polysaccharide shield.

(P-084) Combinatorial glycoform landscapes tune SARS-CoV-2 RBD–ACE2 binding across hosts and variants

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SARS-CoV-2 entry is mediated by binding of the spike receptor-binding domain (RBD) to angiotensin-converting enzyme 2 (ACE2), with interaction strength commonly used to assess susceptibility. Although glycans on ACE2 and spike can modulate this interaction, how glycoform heterogeneity—shaped by host species and biosynthetic context—influences receptor binding remains unclear. Here, we integrated biolayer interferometry with site-specific glycoproteomics to determine how expression system–dependent glycoform landscapes modulate ACE2–RBD interactions across hosts and viral variants. We show that RBD–ACE2 interaction phenotypes are shaped by the combined glycosylation states of viral and host proteins. Whereas RBD binding to human ACE2 remained comparatively stable across variants and expression systems, cervid ACE2 exhibited variant- and expression system-dependent differences in binding affinity. For the Wuhan-Hu-1 RBD, binding to cervid ACE2 was enhanced when the RBD was expressed in 293F cells, whereas CHO-expressed RBD exhibited reduced affinity when paired with 293F-expressed cervid ACE2. In contrast, for the Delta variant, binding affinity decreased when both the RBD and cervid ACE2 were expressed in 293F cells, while pairing 293F-expressed RBD with CHO-expressed cervid ACE2 resulted in higher binding affinity. Binding to the Alpha RBD was comparatively unaffected. Enzymatic deglycosylation of SARS-CoV-2 RBD further revealed that the effects of glycosylation on receptor binding were both variant- and expression system-dependent. Notably, altered glycoform composition at ACE2 N53 and N90, particularly differences in glycan branching and terminal modifications, was associated with changes in binding phenotype, supporting a role for glycosylation as a tunable regulator of SARS-CoV-2 receptor engagement and identifying the biosynthetic origin of both viral and host proteins as an important determinant of receptor-binding phenotypes. Our findings highlight that

incorporating glycoform context into receptor-binding and host-range studies may improve interpretation of zoonotic risk and provide a framework for understanding how glycan heterogeneity shapes virus–host interactions across species.

(P-085) Mammalian soluble lectins as probes for host recognition of zoonotic viruses

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Soluble lectins, as front-line defense proteins in the innate immune system, play a crucial role in host-pathogen interactions by recognizing and binding to cell surface glycans on the microorganisms. However, the glycan specificity of soluble lectins for viral glycans remains poorly understood, particularly for heavily glycosylated viral envelope proteins that facilitate viral entry, immune evasion, host adaptation, and zoonotic spillover.Our research integrates glycobiology and protein biochemistry to develop mammalian soluble lectin-based probes for detecting and characterizing glycans on viral glycoproteins, including rapid changes in glycan composition and site-specific heterogeneity associated with viral pathogenicity and cross-species transmission. We investigate the cooperative roles of representative soluble lectins from three major classes—pentraxins, collectins, and ficolins in glycan recognition during interactions with zoonotic viruses, focusing on influenza A virus and chikungunya virus. By defining the molecular mechanisms governing lectin–glycan recognition, this work will advance our understanding of host–virus interactions and establish a foundation for lectin-based approaches to monitor glycan changes associated with emerging zoonotic threats.

(P-086) Defining Glycan-Encoded Regulation of Glycopeptide-MHCII Stability and Epitope Display

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Antigen presentation by major histocompatibility complex class II (MHCII) molecules is central to $CD4^+$ T cell-mediated adaptive immunity and host defense against infection, cancer, and autoimmune disease. Understanding how antigenic peptides are selected for $CD4^+$ T cell presentation is fundamental to improving health outcomes through vaccines and immunotherapies. While peptide-MHCII complexes are known to be selected based on dissociation kinetics and HLA-DM susceptibility rather than on equilibrium affinity, current models are based primarily on studies of peptide amino acid composition. Emerging evidence demonstrates that MHCII-presented epitopes can carry N-linked glycans that survive endosomal/lysosomal processing with a strong bias towards truncated glycoforms compared to their intact protein precursors, including previous work from our group identifying an HIV ENV-1 MHCII presented glycopeptide (Gpep). The impact of epitope N-linked glycoform identity on glycopeptide-MHCII dissociation kinetics and HLA-DM susceptibility is unexplored, representing a fundamental knowledge gap in the antigen presentation pathway. We hypothesize that N-linked glycans reshape the energetic landscape of glycopeptide-MHCII interactions, thereby encoding distinct glycopeptide dissociation kinetics and susceptibility to HLA-DM-mediated editing, two parameters that influence epitope selection and immunodominance. Here, we aim to establish how N-linked glycoform identity mechanistically regulates glycopeptide presentation by MHCII. Utilizing purified human MHCII and chemoenzymatically synthesized Gpep variants that are unglycosylated, bear defined Man5GlcNAc2, or Man9GlcNAc2 N-glycans, we demonstrate stable MHCII complex formation across all three variants. Current work is quantifying the effect of glycopeptide glycoform on Gpep-MHCII dissociation kinetics in the presence and absence of HLA-DM and defining the molecular mechanisms underlying these effects by integrating mutational analysis of the MHCII peptide-binding groove with all-atom molecular dynamics simulations and structural characterization of the Gpep-MHCII complex. Together, these studies will establish a mechanistic framework for understanding how epitope N-glycoforms influence MHCII antigen presentation and inform the design of vaccines and immunotherapies targeting glycosylated antigens.

(P-087) Quantifying How the Expression System Shapes the Glycan Shield of Viral Glycoproteins

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Viral surface glycoproteins are the main targets of neutralizing antibodies. However, by exploiting the host glycosylation machinery, many of them carry a dense layer of N-linked glycans that masks much of the protein surface from antibody recognition. Individual glycans are highly flexible, so the shield they form is an average over many conformations. It cannot be fully reconstructed from a single static structure. A further complication is that glycan composition is not encoded directly in the viral genome: the same glycoprotein, produced in different cells, can carry different glycoforms at the same glycosylation sites. This is recognized in recombinant antigen production, but it is not yet routinely translated into quantitative, residue-level descriptions of the resulting changes in shielding.To reconstruct the glycan shield we developed GlycoSHIELD: pre-computed glycan conformers are grafted onto the structure of a chosen protein, avoiding the high computational cost of running conventional molecular dynamics simulations for each glycoprotein. Residue-level solvent-accessible surface area (SASA) is rapidly computed with the graphics processing unit (GPU)-accelerated GlycoSASA module, which quantifies the extent to which a given amino acid residue is shielded by glycans.Using this pipeline, we analyse multiple viral glycoproteins, including the SARS-CoV-2 spike, for which experimentally determined site-specific glycosylation profiles are known. For each protein, we compute the shield separately for each expression system, using the site-specific glycan profiles measured in the corresponding cell line, and compare the results sequon by sequon. This isolates the effect of glycan types on accessibility while keeping the protein sequence and conformation fixed. Glycoproteomic data show that glycan maturation depends both on the local protein environment and on the cell line in which the protein was produced. We test whether this translates into shielding: whether sterically constrained sites retain similar shielding regardless of the expression system, while exposed sites differ the most. We also identify epitopes whose accessibility is most sensitive to the choice of expression system. To facilitate biological interpretation, glycan shields mapped onto protein structures are visualised in Mol*.

(P-088) Characterizing Fc-mediated mechanisms for protection against Influenza A or Streptococcus pneumoniae infection

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Microbial pathogens, particularly Influenza A and Streptococcus pneumoniae (S. pneumoniae), represent a consistent public health burden, despite the use of vaccines to prevent infection and severe disease. Fc-mediated pathogen clearance, particularly via effector cell function, offers an important strategy for protection against these pathogens, though the full potential for Fc-mediated mechanisms to provide enhanced responses remains undefined. To address this limitation, we will analyze several Fc-mediated factors, including how N-glycan composition and antibody-epitope location contributes to Fc-receptor engagement and effector function stimulation against cells infected with Influenza A or S. pneumoniae. We will utilize a library of existing antibodies that target different epitopes on these pathogens, evaluating the impact of antibody glycosylation and oligomerization on the Fc-mediated responses. We will quantify the potential of each combination to perform Fc-mediated pathogen clearance in vitro, including through antibody-dependent cell-mediated cytotoxicity (ADCC), using NK cells as effector cells, and target cells that express either Influenza A or S. pneumoniae antigens. Previous work utilizing ADCC has shown an increase in clearance capacity of NK cells upon the addition of antibodies that bind to the target cell. As preliminary work, we have performed ADCC assays using rituximab and Raji cells as the target, and NK cells as effector cells to validate this method is suitable for pathogen clearance quantification. As preliminary preparation, expression of Influenza A or S. pneumoniae antibodies was performed using HEK293 cells, which were then purified, and IgG1 production verified and quantified through SDS-PAGE gels. This work will help establish the optimal combination of Fc-mediated and effector response parameters necessary to maximize immune-related protection against Influenza A and S. pneumoniae.

(P-089) To Fucose or not to Fucose: The divergent glycan recognition of heat-labile enterotoxin and cholera toxin

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Enterotoxigenic Escherichia coli (ETEC) secretes heat-labile enterotoxin (LT), the causative agent of traveler's diarrhea, into the intestinal lumen, where it binds to cell surface receptors, triggering entry into the cell, and cAMP-driven diarrheal symptoms. Epidemiological studies have shown that individuals with blood group A have more severe diarrheal symptoms than individuals with other blood groups infected with LT; however, the molecular basis for this observation requires further elucidation. LT is an AB5 toxin with an enzymatic A subunit and a pentameric B subunit. The B subunit has two binding pockets: a canonical binding pocket with high affinity for GM1 and other gangliosides, and a non-canonical binding pocket that can bind human blood group antigens along with other glycans. Recent findings in CT, a homologous toxin with ~80% sequence identity in the B subunit, have shown that fucosylated glycolipids and glycoproteins recognized by the non-canonical binding pocket play opposing roles in sensitization and protection of the cell from the toxin. This result raises the question of whether fucosylated glycolipids and glycoproteins play similar roles in LT intoxication as they do in CT intoxication. Capitalizing on the methods our team used to study CT, we are employing flow cytometry-based binding assays, analysis of toxin binding to glycoproteins and glycolipids, and an intoxication assay using colon cancer epithelial cell lines with defined glycosylation patterns. In contrast to the results observed with CT, LT preferentially binds to non-fucosylated glycoconjugates. Surprisingly, however, reduced fucosylation does not result in increased LT intoxication, as assayed through measurement of cAMP accumulation. One possible explanation for this result is that LT recognizes non-fucosylated glycolipids that protect the cell from intoxication, similar to our prior observation that fucosylated glycolipids protect cells from CT intoxication. Together, these findings are consistent with a model where toxin binding does not always parallel intoxication. Our current effort focuses on further defining the molecular structure of LT-binding glycoconjugates as well as their divergent roles in LT intoxication. These findings have implications for understanding how changes in cell surface glycosylation may influence susceptibility to different pathogens.

(P-090) N-glycosylation enzyme Mpi Orchestrates Mucin O-glycosylation, Host-Microbe Containment, Paneth Cell defense, and Epithelial Metabolism

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Intestinal homeostasis relies on a protective mucus layer that separates bacteria from the host, with Muc2 as its primary component. This secreted, gel-forming mucin is heavily O-glycosylated, allowing it to retain water and support beneficial bacteria. For the first time, we demonstrate that Muc2 N-glycosylation plays a critical role in mucin maturation, O-glycosylation, barrier integrity, and the prevention of dysbiosis. Using mouse models with hypomorphic and intestinal epithelial-specific N-glycan deficiency due to the loss of the mannose producing enzyme, Mpi- we uncover an unexpected link between N-glycosylation and intestinal homeostasis. Our findings reveal that Mpi hypomorphic mice are highly sensitive to Dextran Sulfate Sodium (DSS)-induced colitis, while Mpiflox; VillinCre mice spontaneously develop disease, exhibiting increased ER stress and dysbiosis. Additionally, electron microscopy, proteomics, and gene expression analyses of goblet and Paneth cells indicate cellular secretion defects, mitochondrial loss, and disruptions in lipid metabolism. These results highlight the fundamental role of N-glycosylation in maintaining intestinal homeostasis.

(P-091) Altered glycan composition of HIV-1 envelope glycoprotein enables virus escape from broadly neutralizing antibodies

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Envelope glycoprotein (Env), a trimer of gp120 and gp41 dimers, is critical for HIV-1 infectivity and immune recognition. N-glycans contribute >50% to gp120 molecular mass and form discreet glycan-microdomains. gp120 glycosylation is variable due to the diversified Env sequences and alternative potential glycosylation sites (NGS) in immune-escape variants that evade Env-specific broadly neutralizing antibodies (BnAbs). However, the impact of glycan composition on BnAbs activity is not well understood. To better define the role of Env glycan microdomains, we tested the activity of BnAbs against Env-pseudotyped viruses with altered gp120 glycosylation.We used Env from an immune-escape virus WEAU-d391 (WT) and perturbed glycan microdomains using site-specific mutagenesis and cell-specific glycosylation. Env-pseudotyped viruses were produced in FreeStyle 293-F [293F (FS); +/- □-mannosidase 1 inhibitor], Expi293F (XP), and Expi293F-GnT1- (XP-GnT1-) cells. These Envs were used: WT, CD4-binding-site (CD4bs)-Mut (N276D+N463D), N188-Mut (N188D), and 3NGS-Mut (N188D+N276D+N463D). Virus infectivity and BnAbs activity were assessed using TZM-bl reporter cells. We used BnAbs targeting CD4bs (VRC01), apex (PGT145, PGDM1400), and C3 region (PGT122). PGT122 was a control BnAb (minimally impacted by glycan variations). Recombinant gp120 trimers (rgp120) of WT and CD4bs-Mut were produced in FS and XP cells and profiled for total N-glycans and site-specific N-glycan heterogeneity. Structural modeling was used to interpret the glycomics and functional data.Regardless of the producing cells, NGS modifications did not abolish the ability of virus to infect the reporter cells, but viruses with Envs without N188 had reduced infectivity. Compared to WT, Envs without both N276 and N463 were ~15-fold more sensitive to VRC01, whether produced in FS or XP cells. Moreover, VRC01 exhibited increased neutralizing activity against WT produced in XP-GnT1- but not in FS in the presence of □-mannosidase 1 inhibitor, indicating that the XP-GnT1- cells produced smaller glycans that enabled VRC01 access. FS-WT virus was neutralized by apex BnAbs (PGT145, PGDM1400) effectively, whereas FS-N188-Mut virus was >80-fold less sensitive, suggesting that N188 glycan played an important role in this recognition. All XP-viruses were less sensitive to apex-BnAbs whereas all XP-GnT1- viruses were more susceptible to the same BnAbs. PGT122 neutralized Env variants similarly, except Envs without N188 produced in XP-GnT1-. XP- vs. FS-produced Env had more high-mannose and less complex glycans, suggesting a cell-specific differential glycosylation of Env. Env N160 glycans from XP vs. FS cells had more Man5 and less Hex5HexNAc4Fuc1 glycans.Altered Env glycan-microdomains impacted HIV-1 infectivity and sensitivity to apex-targeting BnAbs. Some Env glycoforms enabled escape from apex-BnAbs whereas others enhanced BnAbs sensitivity. These findings will inform design of future therapeutic approaches.

(P-092) Oxygen-dependent glycosylation of Skp1 regulates F-box protein selection and abundance in the unicellular parasite Toxoplasma gondii and the social amoeba Dictyostelium discoideum

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The Skp1/cullin-1/F-box protein class of E3 Ub ligases mediates proteasomal degradation of a broad swath of the proteome. In many protists, the Skp1 adaptor, which assembles high affinity subcomplexes with F-box proteins (FBPs) that serve as substrate receptors and reversibly docks with Cul1, are regulated by a novel posttranslational modification of their Skp1 adaptors. A specific proline in its intrinsically disordered C-terminal region (CTR) is subject to oxygen-dependent hydroxylation, which is in turn acted upon by 5 glycosyltransferases to generate a relatively ordered pentasaccharide. Genetic blockade of hydroxylation or glycosylation is tolerated but alters oxygen-sensing behaviors of the social amoeba Dictyostelium and the human pathogen Toxoplasma gondii. Biophysical studies indicate that glycosylation interferes with a charge-based fuzzy interaction contributed by the CTR to Toxoplasma Skp1 homodimerization, which is competitive with FBP interactions owing to a shared interface. Furthermore, the profile of FBPs associated with Skp1 varies between wild-type and mutants that cannot glycosylate Skp1, or between oxygen replete and oxygen-limited cells, of both species based on proteomic analysis of co-immunoprecipitates by mass spectrometry. Direct examination shows that the steady state level of some affected FBPs is reduced in a proteasome-dependent manner in the absence of Skp1 modification. Given that NMR-guided molecular dynamics simulations suggest that the folded glycan influences CTR organization in a manner favorable for FBP interactions, Skp1 glycosylation may be stabilizing. To examine this further, several FBPs were overexpressed in

wild-type Dictyostelium to more directly test Skp1 isoform preference. Whereas FbxN strongly preferred fully glycosylated Skp1 as its partner, FbxF strongly preferred unmodified Skp1, with others in between. To test the significance of its preference, FbxN was expressed in prolyl hydroxylase-KO amoebae that cannot modify Skp1. FbxN was poorly expressed, aggregated and partially degraded. This contrasted with the expected 1:1 complex formed with glycosylated Skp1 based on gel filtration and mass photometry. Thus the Skp1 glycan both regulates its dimerization, which sequesters Skp1 from FBP complexes, and prioritizes interaction with select FBPs. Both effects are thought to involve conformational constraints on its intrinsically disordered C-terminal domain. By implication, glycosylation exerts a selective effect on the cellular proteome, and mediates a novel yet widespread mechanism of oxygen sensing in unicellular organisms that is critical for optimal adaptations to their local environments.

TOPIC 11: Immunology, Autoimmunity, and Glycoimmunology

(KEY11-001) Engineered endoglycosidases selectively target afucosylated IgG and suppress pathogenic antibody effector functions

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IgG antibodies are critical mediators of protective immunity whose biological activities are governed by the conserved N-glycan attached to Asn297 within the Fc region. Among Fc glycoforms, afucosylated IgG exhibits enhanced affinity for an activating Fc γ receptor (FcγR) and drives potent effector responses, including antibody-dependent cellular cytotoxicity (ADCC) and inflammatory cytokine release. Although afucosylated IgG represents only a minor fraction of circulating antibodies in healthy individuals, elevated levels of antigen-specific afucosylated IgG have been strongly associated with disease severity and pathology in infectious and immune-mediated disorders, including dengue virus infection, COVID-19, and alloimmune cytopenias, making afucosylated IgG an attractive target for precision immunomodulation. Recently, we identified a family of compact single-domain IgG-specific endoglycosidases from Corynebacterium species (CU43) that selectively hydrolyze the conserved Fc N-glycan, thereby suppressing Fc-mediated effector functions while preserving antigen recognition. Here, we report the engineering of a CU43 variant, CU43-NRW, that exhibits remarkable selectivity for afucosylated IgG glycoforms, enabling preferential targeting of pathogenic afucosylated antibodies while sparing the predominant pool of circulating fucosylated IgG. Using an integrated approach combining protein engineering, biophysical analyses, and structural studies, including a cryo-EM structure of CU43-NRW in complex with afucosylated human IgG1 Fc, we define the molecular determinants that enable discrimination between highly similar fucosylated and afucosylated Fc glycans. These studies reveal how engineered alterations within the enzyme remodel glycan recognition and generate unprecedented glycoform selectivity. To determine the biological relevance of this selectivity, we evaluated CU43-NRW in humanized FcγR mouse models using afucosylated and fucosylated anti-CD4 and anti-CD20 antibodies. CU43-NRW selectively prevented T and B-cell depletion mediated by afucosylated antibodies while having minimal effects on the corresponding fucosylated antibodies, demonstrating that glycoform-selective antibody silencing can be achieved in vivo. These findings establish CU43-NRW as a unique platform for the selective modulation of pathogenic antibody responses and provide the first mechanistic framework for glycoform-selective IgG deglycosylation. Collectively, this work provides proof-of-concept for a precision therapeutic strategy that selectively neutralizes disease-driving afucosylated antibodies while preserving the majority of protective humoral immunity. Furthermore, it establishes a foundation for the rational development of next-generation glycoform-selective enzymes as a new therapeutic paradigm for the selective silencing of pathogenic antibodies in antibody-mediated diseases.

(PT-015) IgM present in Circulating Immune Complexes from IgA Nephropathy Patients Directly Binds to the Tn Antigen Within IgA1 and Is Mitogenic to Mesangial Cells

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IgA nephropathy (IgAN) is the most prevalent type of glomerulonephritis globally (at least 2.5/100,000), contributing to end-stage

renal failure in approximately 30-40% of those affected. The prevalence of IgAN is likely under-represented, as early diagnosis without biopsy is often missed. Most cases of IgAN are sporadic; however, a small number are associated with family clustering. The defining feature of IgAN pathogenesis is the deposition of galactose-deficient IgA1-containing circulating immune complexes (CICs) in the renal mesangium, leading to disease pathology. However, the molecular mechanism of IgAN pathogenesis remains largely unclear. Prior, we purified and characterized several megadalton-sized anti-Tn CICs containing Gd-IgA1, together with IgM and variable amounts of IgG, important in IgAN pathogenesis. Here we have thoroughly dissected the role of each antibody present in anti-TnCICs. We purified IgM, IgA, and IgG to apparent homogeneity and demonstrated, contrary to prior assumptions in the field, that IgM, not IgG, directly interacts with Gd-IgA1. Interestingly, our work showed that human renal mesangial cells (HRMCs) express cryptic Tn antigen and purified anti-Tn IgM from CICs directly binds to such Tn antigen expressed on many cell-surface glycoproteins by HRMCs, but not to purified IgG or Gd-IgA1. This provides the first clue regarding how CICs deposit in the kidneys. IgM interaction to HRMCs is mitogenic which can be efficiently inhibited by hapten sugar (Di-GalNAc). Collectively, these results provide the key players of IgAN pathogenesis and the possible strategy to diminish CICs formation and the deleterious impact on HRMCs stimulation.

(PT-016) The C-type Lectin and Hyaluronan Receptor Layilin Regulates IL10 Signaling in Macrophages

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More than 3 million Americans are affected by inflammatory bowel disease (IBD), a group of chronic, relapsing disorders characterized by intestinal mucosal injury and persistent inflammation. Although the initiating causes of IBD remain incompletely understood, disease progression involves dysregulated interactions among immune cells, nonimmune cells, and the intestinal extracellular matrix (ECM). Hyaluronan (HA), an abundant ECM glycosaminoglycan with important immunoregulatory functions, accumulates in inflamed intestinal tissues from patients with IBD and contributes to leukocyte recruitment and activation. Layilin, a transmembrane receptor containing a C-type lectin-like domain that can bind HA, is transcriptionally reduced in IBD tissues, and systemic loss of Layilin exacerbates experimental colitis in mice. These observations suggest that Layilin restrains intestinal inflammation, but the underlying cellular and molecular mechanisms remain unknown.We hypothesized that loss of Layilin promotes a hyperinflammatory macrophage phenotype by impairing signaling through the anti-inflammatory interleukin-10 receptor (IL-10R). Following induction of acute colitis with 2% dextran sulfate sodium, Layilin-deficient mice exhibited increased disease severity, intestinal permeability, and inflammatory macrophage accumulation. Layilin-deficient macrophages were hyperresponsive to LPS-induced stimulation, producing significantly increased levels of pro-inflammatory cytokines including TNF and IL-6. Consistent with these findings, colonic proinflammatory cytokine levels were elevated in layilin deficient mice compared to controls during acute colitis and remained markedly increased after five days of recovery, whereas colonic IL-10 levels were similar between groups. Transcriptional analysis of murine colon tissue revealed LAYN deletion dramatically alters transcriptional programs during intestinal inflammation including strong upregulation of multiple immune signaling networks including: TLR-MyD88-NF-kB signaling, Chemokine networks, Type I/II interferon response genes, and pattern-recognition receptors, suggesting that layilin normally functions to restrain immune activation.Flow-cytometric analysis of splenic, colonic, and bone marrow-derived macrophages demonstrated that loss of Layilin was associated with reduced IL-10R expression across macrophage activation states. Layilin-deficient macrophages also exhibited impaired IL-10-induced STAT3 signaling, providing a potential mechanism for their failure to acquire an inflammation-resolving phenotype. Together, these findings identify Layilin as a previously unrecognized regulator of macrophage IL-10 responsiveness and suggest that reduced Layilin expression may contribute to persistent intestinal inflammation by disrupting macrophage-mediated resolution. The Layilin-IL-10R signaling axis may therefore represent a potential therapeutic target for promoting inflammatory resolution in IBD.

(PT-017) Inhibition of O-glycosylation on CD-antigens hampers immunological synapse formation and T-cell activation

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Immune cells display a wide variety of glycoproteins on the glycocalyx, which are directly involved in the formation of an immunological synapse between the cognate T-cell receptor (TCR) on T-cells and the peptide-loaded major histocompatibility complex (pMHC) on the antigen-presenting cells (APC). Selected group of CD antigens (e.g., CD43, CD45, CD93, and CD162) on the immune cell surface are heavily *O*-glycosylated (20-80 *O*-glycosites) and are known as long surface molecules (LSMs) due to their projection length of 40-50 nm. Studies by van der Merwe, Germain, Sperling, Baum, and others have shown that LSMs, particularly CD43, is excluded from the central supramolecular activation cluster (cSMAC) during APC-T-cell interactions in order to establish the immune synapse. Studies on immune synapse have focused on TCR-pMHC interactions, cytoskeletal rearrangements, kinase cascades, gene expression, and cytokine secretion. The functional roles of *O*-glycosylation of CD antigens in the context of the immune synapse have not been explored.

We hypothesized that proper *O*-glycosylation and the glycan load on CD43 are critical for its readiness to move away from the pMHC-TCR contact point, and that any disruption of *O*-glycosylation could interfere with immune synapse formation. We employed the peracetyl *N*-thioglycolyl-D-galactosamine (Ac_5GalNTGc, **Tg**) [*Ref*: Wang, S. S., *et al. Cell Chem Biol*. **20**, 699-710 (2021)] as a tool to probe the functional consequences of *O*-glycosylation inhibition. The extracellular domain of CD43 ($CD43_{ex}$) is estimated to carry 80-90 *O*-glycans, which impart a rigid bottle-brush-like structure on the glycocalyx. To profile the *O*-glycosites, we expressed $CD43_{ex}$-Fc-His in Jurkat cells (human T-lymphoma) *via* lentiviral transduction. $CD43_{ex}$-Fc-His was purified, subjected to digestion using trypsin, Glu-C, and StcE mucinase, and the glycopeptides were analyzed by nano-LC-MS/MS. Results showed that, on average, 70 *O*-glycosites were occupied in $CD43_{ex}$-Fc-His; this was reduced to 40 *O*-glycosites upon treatment with **Tg**, accompanied by metabolic incorporation of *N*-thioglycolyl-D-galactosamine (GalNTGc).

We studied the effect of *O*-glycosylation inhibition on T-cells using three in vitro models: (a) rearrangement of actin cytoskeleton of Jurkat cells upon activation with anti-CD3 (UCHT-1), (b) Co-culture of Jurkat cells with staphylococcus enterotoxin E (SEE)-treated Raji-cells, and (c) mouse primary T-cells cultured with primary macrophages pulsed with the OVA-1 peptide. Our results show that inhibition of *O*-glycosylation using **Tg** hampered the immune synapse formation, prevented the formation of a smooth actin ring from the microvilli, and reduced T-cell activation, as revealed by CD69 and IL-2. These results highlight the importance of *O*-glycosylation in regulating dynamics of glycoproteins on the plasma membrane and cytoskeletal reorganization in the context of immune homeostasis, vaccine response, and autoimmune disorders.

(P-093) The Quantification and Characterization of Galactose Deficient IgA1 in IgA Nephropathy

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IgA nephropathy (IgAN), also known as Berger disease, is the most common form of primary glomerulonephritis worldwide and accounts for 20–40% of end-stage renal disease within one to two decades of diagnosis. IgAN is characterized by a variety of clinical presentations including hematuria and proteinuria. Due to a lack of mechanistic understanding of this disease, however, there are no accepted biomarkers nor therapies specific for monitoring and treating IgAN. A widely accepted feature of IgAN is the aberrant O-glycosylation of the hinge region (HR) of IgA1. The standard glycosylation profile of the HR contains predominantly the galactosylated T-antigen (Galβ1-3GalNAcα1-Ser/Thr). However, a subpopulation of IgA1 exhibits a galactose-deficient (Gd) O-glycan, exposing the Tn antigen (GalNAcα1-Ser/Thr). The current "four-hit" hypothesis for onset of IgAN proposes that increased production of Gd-IgA1, formation of autoantibodies towards the Gd-IgA1, generation of circulating immune complexes (CICs), and mesangial deposition lead to inflammation and the progression of the disease. However, the biochemical understanding of IgAN, including the molecular composition, formation, and pathogenic activity of these CICs, remains poorly understood. Importantly, current methods for quantifying Gd-IgA1 are highly assay-dependent and fail to account for its heterogeneity or its incorporation into CICs, limiting its utility as a diagnostic biomarker. In this work, we have successfully developed an isolation strategy using immobilized Helix pomatia agglutinin (HPA) in an affinity chromatography approach. This allows the separation of distinct IgA1 O-glycoforms and enrichment of HPA+ (Gd-IgA1) glycoform. Glycosidase treatments and lectin blotting provide additional evidence that this approach promotes the selective isolation of the Gd-IgA1 glycoform, hypothesized to be a key biomarker of IgA nephropathy (IgAN). Furthermore, this method enables direct quantification of specific glycoforms of IgA1 and provides a valuable

step for assessing IgA1 O-glycan heterogeneity in sera from healthy donors and IgAN patients, while facilitating further biochemical investigation into the role of Gd-IgA1 in pathogenic IgAN CICs.

TOPIC 11: Immunology, Autoimmunity, and Glycoimmunology

(P-094) Investigating the effects of various proinflammatory cytokines on endothelial cells' ability to sialylate IgG

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The endothelium is a dynamic structure, actively fine-tuning the immune landscape that surrounds it. Recycling of IgG antibodies via the FcRn-dependent pinocytosis mechanism by endothelial cells is critical for the maintenance and establishment of humoral immunity. Glycan composition at the conserved N-glycosylation site N297 within the Fc CH2 domains alters the effector function of IgG by skewing downstream Fc receptor binding. The glycosyltransferase ST6Gal1 adds a terminal α2,6-sialic acid to the Fc glycan, thereby resulting in an antibody that exhibits a decreased ability to elicit proinflammatory effector functions. Data linking decreased IgG terminal sialylation and galactosylation to inflammation and the severity of inflammatory disease have been compiled for decades. Recently, we published our findings, showing that endothelial cells can sialylate IgG in vitro and that the loss of the FcRn-mediated recycling pathway results in a significant reduction in IgG sialylation in vivo. Within that study, IFN-γ stimulation of endothelial cells resulted in the decreased ability of endothelial cells to sialylate IgG in vitro. However, the effects that other inflammatory cytokines may have on the endothelium's ability to sialylate IgG have not been investigated. Here we report on a broader array of proinflammatory cytokines as modulators of the IgG recycling and sialylation pathway in endothelial cells in vitro. The ongoing goal of this project is to understand how these inflammatory signals control endothelial cell-mediated IgG sialylation to shape antibody-mediated immunity and homeostasis.

(P-095) The Uniquely Unfavorable E430 and E345 Residues Inhibit Immunoglobin G1 Hexamer Formation Through Distinct Mechanisms

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Immunoglobulin G1 (IgG1) antibodies exhibit the ability to form hexameric oligomers on a target cell surface following antigen engagement, promoting recruitment of protective molecules including the C1 component of complement. The E345K and E430G variants of the IgG1 crystallizable fragment (Fc) reveal enhanced oligomerization and complement activation following target binding compared to the wild type IgG1, though how these structural modifications trigger the seminal event of IgG oligomerization remain undefined. We evaluated structural features and motion of the Fc domains from wild-type IgG1 Fc as well as the E345K and E430G variants using solution NMR spectroscopy and molecular dynamics simulations. Advanced expression and isotope-labeling strategies facilitated high resolution NMR of the Fc domain, capturing chemical shifts over a wide range of conditions as well as motion through pseudo-3D experiments that quantify the spin relaxation rates R1 and R2. We identified Fc regions that exhibited increased motion in both variants and wild type, including the N-terminal hinge, CH2-CH3 connecting loop and the C-terminus. The hexamer-promoting variants additionally revealed the presence of slightly increased Fc oligomerization. These variants demonstrated significant chemical shift perturbations and differences in hydrogen bond formation relative to the wild type Fc. Furthermore, we observed increased conformational flexibility of the CH2 domains relative to the CH3 domains in contrast to the wild type that showed similar motion regimes across both domains. Molecular dynamics simulations of the Fc showed comparable increased interdomain motions of the hexamer-promoting variants relative to the wild type IgG1 Fc. We hypothesize that wildtype IgG1 Fc exhibits limited interdomain motions, with hexamer-promoting conformations disfavored in the conformational ensemble. The E345K and E430G substitutions increase sampling of hexamer-competent conformations, promoting hexamer formation.

(P-096) A Semenogelin-Derived Glycopeptide Bound to a Galectin-3 N-Linked Glycan Complex on the Sperm Surface is the Potential Target of the Human Contraception Antibody

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The human contraception antibody (HCA) is a non-hormonal contraceptive monoclonal antibody that functions by agglutinating and immobilizing sperm. To date, the HCA epitope has been described as an N-linked polylactosamine glycan motif on CD52, but this assignment has been inferred only by colocalization with anti-CD52 and S19 antibodies in western blots. Here, we report that HCA may instead bind semenogelin-galectin-3 complexes associated with the sperm surface. Semenogelins are gel forming, highly glycosylated proteins, with molecular weights of 50-70 kDa that are cleaved into smaller peptides that adhere to sperm during semen liquefaction. In this study, we isolated the HCA antigen from seminal plasma and sperm using an HCA affinity column and identified semenogelin by mass spectrometry. Western blot analysis of seminal plasma showed that HCA binds to a large smear of proteins with a higher apparent molecular mass than CD52, and that these may correspond to semenogelin sequences. Flow cytometric analysis of N-glycanase deglycosylated sperm demonstrated that HCA binding requires sperm surface N-linked glycans. However, despite this apparent glycan dependence, HCA failed to bind any glycans presented on a comprehensive glycan microarray. In addition to semenogelin, galectin-3 was identified in the HCA purified sperm lysates, supporting a model in which HCA recognizes semenogelin-derived glycopeptides that are tethered to the sperm surface via a galectin-mediated interaction with an N-linked glycan. We found that O-linked glycan modifications on the semenogelin proteins contain LacNAc motifs that can bind to galectins. Finally, depletion of galectins bound to the sperm surface by competitive inhibitors reduced HCA-mediated agglutination, and this could be rescued by addition of recombinant galectin-3/semenogelin-2 complexes to sperm cultures. Collectively, our findings suggest that HCA's binding site may involve semenogelin-galectin-3 complexes that are tethered to the sperm surface via N- and O-linked glycans.

(P-097) Elucidating the role of heparan sulfate in macrophage homeostasis by transcriptomics

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Heparan sulfates (HS) are linear, sulfated glycosaminoglycans that bind a broad range of HS binding proteins, contributing to growth factor and cytokine signaling and extracellular matrix formation. In macrophages, these HS-protein interactions can influence inflammatory activation, chemotactic responses, and cytokine signaling, which positions HS as an important component of the macrophage response to extracellular factors. Beyond inflammation, it remains unclear whether HS plays additional roles in macrophage function. In this study, we therefore sought to measure genome-wide transcriptional differences between WT and HS-deficient BV2 macrophages. EXT1 KO macrophages, which lack an essential glycosyltransferase for HS backbone elongation were derived using CRISPR-Cas9 knockout. WT and EXT1 KO macrophages were then subjected to bulk RNA sequencing, differential gene expression analysis, and gene set enrichment analysis (GSEA). Compared with WT cells, EXT1 KO cells exhibited 947 differentially expressed genes, including 703 downregulated and 244 upregulated genes. GSEA revealed coordinated negative enrichment of TNF–NF-κB, type I and type II interferon, IL-6–JAK–STAT3, cytokine signaling, and innate immune pathways in EXT1 KO macrophages compared to WT macrophages. In contrast, cholesterol and steroid biosynthetic pathways exhibited significant positive enrichment in EXT1 KO cells. Genes involved in the mevalonate pathway, squalene and lanosterol synthesis, and post-lanosterol cholesterol biosynthesis were consistently upregulated, suggesting coordinated activation of the sterol biosynthetic program. Collectively, these results suggest a dual role for HS in macrophages, supporting inflammatory signaling programs while restraining sterol biosynthetic pathways.

(P-098) Sialic Acid 9-O-Acetylation Maintains Tolerance Against Autoimmune Arthritis

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Sialic acid (Sia) on N-linked glycans promotes immune tolerance by engaging Sia-recognizing immunoglobulin superfamily lectins (Siglecs) to trigger inhibitory signals in immune cells. Siglec engagement is nullified by 9-O-acetylation (9-O-Ac) of terminal Sia residues. Several glycoenzymes control Siglec-ligand availability: ST6Gal1 and SIAE both increase ligands by adding α2,6-linked Sia or removing 9-O-Ac, respectively, while CASD1 decreases ligands by adding 9-O-Ac. However, the functional consequences and interplay of these enzymes in autoimmunity remain poorly understood. Based on their known functions, we hypothesized that ST6Gal1 and SIAE would maintain tolerance and prevent autoimmunity, while CASD1 would compromise tolerance. We employed the collagen-induced arthritis (CIA) model, in which mice were immunized with chicken type II collagen (CII) in complete Freund's adjuvant. This regimen breaks tolerance in ~30% of WT C57BL/6 mice, allowing us to evaluate both increases and decreases in pathology. Unexpectedly, we found no difference in arthritis incidence or severity in mice lacking ST6Gal1 compared with WT controls. CII-specific IgG from ST6Gal1 KO mice lacked detectable α2,6-linked Sia and instead possessed aberrant α2,3-linked Sia, which may explain the unchanged clinical phenotype. Likewise, we observed no difference in clinical arthritis in SIAE KO mice compared with WT controls. Furthermore, mice lacking the Siglecs CD22 or Siglec-G, which bind α2,6-Sia or α2,3-/α2,6-Sia, respectively, also developed arthritis at comparable incidence and severity to controls, suggesting redundancy among Siglecs or a possible role for a different receptor. Contrary to our hypothesis, 100% of CASD1 KO mice developed severe arthritis. Anti-CII IgG titers were equivalent across all strains indicating that the observed phenotypes were unrelated to autoantibody quantity. Instead, we found that CII-specific IgG from WT mice possesses 9-O-Ac Sia, while this modification is absent in CASD1 KO mice. Because CASD1 acts on donor Sia upstream of all sialyltransferases, its deletion strips 9-O-Ac from every sialoglycan irrespective of linkage, escaping the redundancy that may mask single-enzyme and single-Siglec KO phenotypes. The inversion of our prediction suggests that the functionally dominant Siglec-bearing cells in this model are regulatory rather than effector: by blocking Siglec engagement, 9-O-Ac shields regulatory populations from inhibition and thereby preserves tolerance. Thus, these findings identify 9-O-Ac Sia as an unrecognized brake on autoimmunity and reveal a potential pathway for therapeutic modulation of autoimmune disease.

(P-099) Defining the impact of combined cytokine and N-glycan modifying treatments on the potency of NK cell ADCC

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Antibody-dependent cell mediated cytotoxicity, or ADCC, is a process whereby effector cells are recruited to antibody coated surfaces to induce target cell death. Fc γ receptors, namely FcγRIIIa/CD16a that are expressed on cytotoxic natural killer (NK) cells (CD56dim CD16+), are the primary mediators of ADCC. ADCC is targeted for cancer immunotherapy to treat solid tumors and hematological cancers, whereby increasing the potency is expected to improve patient outcome. ADCC potency may be increased through exogenous cytokine treatment, specifically IL-2 and IL-15. IL-2 and IL-15 both demonstrate an increase in ADCC potency in NK cells, but the toxicity of IL-2 in high doses and the low half-life of circulating IL-15 reduce their feasibility as standalone treatments. With these limitations, determining other methods for increasing ADCC is crucial. Our laboratory previously determined that CD16a N-glycosylation mediates antibody binding affinity and ADCC potency, with oligomannose N-glycans resulting in higher levels of ADCC compared to complex-type N-glycans. Of the five N-glycosylation sites on CD16a, the glycan on N162 alone has been determined to regulate antibody binding affinity. Our goal here is to evaluate to the impact of combining the modulation of glycan composition through kifunensine treatment with cytokine treatment. These analyses are performed using ex vivo primary human NK cells. Our results show that a combination treatment of IL-2 and kifunensine or IL-15 and kifunensine increases ADCC.

Pretreating primary NK cells with a combination of IL-15 and kifunensine resulted in increased ADCC potency in four out of five donors when compared to the no cytokine control. Two out of those four donors have ADCC levels higher than IL-15 or kifunensine individually. A pretreatment of IL-2 and kifunensine resulted in increased ADCC in all three donors, with two donors having ADCC levels higher than IL-2 or kifunensine alone. This study demonstrates that both cytokines and N-glycan composition synergistically increase ADCC potency, providing a new strategy improve ADCC and through these studies promote a greater understanding of ADCC regulation.

(P-100) Immune Complexes Consisting of Galactose-deficient IgA1, IgG Autoantibodies, and Complement C3 Activate Cultured Primary Human Mesangial Cells

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IgA nephropathy (IgAN) is an immune complex-associated mesangioproliferative glomerulonephritis. The glomerular immune-complex deposits, comprised of IgA1 with some O-glycans deficient in galactose (galactose-deficient IgA1; Gd-IgA1), Gd-IgA1-specific IgG autoantibodies, and complement C3, likely originate from the circulation. Elevated C3-IgA colocalization in glomerular immune-complex deposits is associated with mesangial hypercellularity. We have previously shown that Gd-IgA1 added to IgAN-patient serum is bound by existing IgG autoantibodies to form new immune complexes with activated C3. However, it is unknown which form of activated C3 can support mesangioproliferative activity of Gd-IgA1-IgG immune complexes. To address this gap in knowledge, we analyzed the size, composition, and biological activity of immune complexes formed from recombinant polymeric Gd-IgA1 and recombinant IgG autoantibodies in C3-depleted serum of healthy individuals and supplemented with either C3 or C3b.Immune complexes were formed in vitro from recombinant polymeric Gd-IgA1 and recombinant IgG autoantibody in C3-depleted serum supplemented with either C3 or C3b and isolated by size-exclusion chromatography as fractions F1-F8 with molecular mass ranging from ~4 MDa to 0.7 MDa. C3-depleted serum served as a negative control. Biological activity of immune complexes in the chromatographic fractions F1-F8 was determined using click-iT EdU cell-proliferation assay and cultured primary human mesangial cells. Composition of immune complexes was analyzed by ELISA (total IgA and IgG-IgA, C3-IgA, and C3-IgG immune complexes). To detect IgA- or IgG-containing immune complexes with activated C3, we used mAb 3E7 for capture (binds activated C3, C3b, and iC3b, but not C3 with intact thioester bond) followed by IgA or IgG detection.Immune complexes formed in C3- or C3b-repleted sera had elevated IgA concentrations in fractions F6-F8. Immune complexes in C3-repleted serum had proliferation-inducing activity in F6-F8. Immune complexes in C3b-repleted serum had a proliferation-inducing activity in F5 and F8. Individual purified proteins (Gd-IgA1, IgG, C3, or C3b) had little or no proliferation-inducing activity. IgG-IgA immune complexes were detected in F3-F8 of C3- and C3b-repleted sera. C3-IgA and C3-IgG immune complexes were detected in F3-F8 of C3- and C3b-repleted sera, with lower amounts in F5-F8 of C3b-repleted serum. Immune complexes with activated C3 and IgA were found in greater amounts in C3-repleted vs. C3b-repleted serum.The results from proliferation and binding assays indicated that Gd-IgA1-IgG immune complexes required binding of activated C3 to induce proliferation of cultured primary human mesangial cells. Activated C3 and C3b provided similar functions, although they differentially impacted the size and amounts of the resultant immune complexes.

(P-101) Galactose-Deficient IgA1-Containing Immune Complexes from Sera of Patients with IgA Nephropathy, but not the Individual Protein Components, Activate Cultured Primary Human Mesangial Cells

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IgA nephropathy (IgAN) is a common primary glomerulonephritis characterized by the glomerular immune-complex deposits containing IgA, IgG, and complement C3. IgA is exclusively of IgA1 subclass and enriched for glycoforms with some O-glycans deficient in galactose (galactose-deficient IgA1; Gd-IgA1). IgG in the glomerular immune-complex deposits is enriched for IgG autoantibodies specific for Gd-IgA1. C3 is colocalized with IgA and, to a lesser degree, with IgG; a greater colocalization of C3

with IgA correlates with active kidney injury including mesangial hypercellularity. These glomerular Gd-IgA1-containing immune-complex deposits likely originate from the circulation. In this study, we isolated immune complexes from sera of IgAN patients and determined their size, composition, and biological activity.Immune complexes were isolated from sera of 6 IgAN patients by size-exclusion chromatography as fractions F1-F8 (molecular weight ranged from ~4 MDa to 0.7 MDa). Composition of immune complexes was analyzed by ELISA, immunoglobulin multiplex assay, and blue-native gel electrophoresis or SDS-PAGE followed by immunoblotting with antibodies specific for IgA, IgG, and C3. Biological activity was tested using cultured primary human mesangial cells. Cytokines were quantified using magnetic bead-based multiplex immunoassays (n=5) and mesangial-cell proliferation was determined by click-iT EdU cell-proliferation assay (n=3). Fractions F1–F8 derived from IgA-depleted sera served as negative controls (n=2).High-molecular-mass immune complexes (~2 MDa) eluted in fractions F4-F5, followed by low-molecular-mass immune complexes (~1 to 0.7 MDa) in fractions F6-F8. The abundance of IgA, IgG, and C3 in these fractions varied among IgAN patients, but in general the amounts were highest in F8. Covalent association of C3 with IgA and/or IgG was confirmed in fractions F4-F8 from native sera, but not in those from IgA-depleted sera. C3 molecular forms included C3(H2O), C3b, and iC3b. Functionally, fractions F8 from native IgAN sera (n=5) added to mesangial cells increased production of IL-6, IL-8, and MCP-1 by 5- to 15-fold (p<0.001 for all); IgA depletion reduced this activity. IgA concentration correlated with production of IL-6 (p=0.0003), IL-8 (p=2.86e-06), and MCP-1 (p=0.0003). Fractions F8 (n=3) stimulated mesangial cell proliferation, whereas other fractions showed donor-dependent stimulatory or inhibitory effects. Individual purified proteins, Gd-IgA1, IgG autoantibody, C3, C3a, C3b, or iC3b did not significantly increase cellular proliferation of mesangial cells or production of IL-6, IL-8, and MCP-1.In summary, low-molecular-mass Gd-IgA1-IgG-C3-containing immune complexes from sera of IgAN patients, but not their individual components, activated mesangial cells and induced overproduction of cytokines/chemokines that accentuate disease activity in IgAN.

(P-102) Adjuvants control protective humoral immunity to intracellular bacteria through IgG Fab sialic acid O-acetylation

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Antibodies are central to vaccine-induced protective immunity but have traditionally been considered to have limited efficacy against intracellular pathogens. Several licensed vaccines against intracellular pathogens are designed to recapitulate infection-associated TH1 immunity, yet have failed to generate protective antibodies. Using Listeria monocytogenes (Lm), we recently found that TH1 polarization drives CASD1-mediated 9-O-acetylation of sialic acid on IgG Fab N-glycans, rendering antibodies non-protective, whereas disrupting TH1 signaling or promoting TH2 responses prevents acetylation and restores protection. Here, we show that rational adjuvant selection for protein-based prime-boost vaccination elicits highly protective antibodies by supporting TH2-driven suppression of CASD1 and IgG sialic acid acetylation. Priming with ActA, a key Lm virulence factor, plus the TLR9 agonist CpG results in IFNg-producing T follicular helper cells (TFH) and increased CASD1 expression in plasma cells (PC) in draining lymph nodes. Conversely, priming with ActA formulated with TH2-promoting adjuvants like Alum or complete Freund’s adjuvant yields IL-13-producing TFH cells and low CASD1 expression in PC. Moreover, blockade of TH2 cytokine signaling increases CASD1, indicating active suppression of CASD1 during B cell responses. Importantly, TH1 immunization generates ActA-specific antibodies containing 9-O-acetylated sialic acid, whereas TH2 immunization generates antibodies lacking acetylation. Adoptive transfer of ActA-specific antibodies generated under TH2 conditions provides robust protection against lethal Lm infection in recipient mice, and this protection requires CD22 receptor engagement with IgG Fab sialic acid. Together, these findings show that TH2-biased adjuvants generate protective antibodies by suppressing CASD1-mediated sialic acid acetylation on IgG Fab N-glycans, identifying adjuvant-directed glycan programming as a strategy to improve humoral immunity against intracellular pathogens.

(P-103) O-Glycosylated CD43 is a novel pan-immune checkpoint in myeloid leukemia

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Aberrant glycosylation is a hallmark of cancer cell, remodeling immune recognition through mechanisms that remain incompletely defined in hematologic malignancy. In acute myeloid leukemia (AML), CD47 was identified as a promising "don't eat me" immune

checkpoint, yet despite strong preclinical rationale, CD47-SIRPα blockade with magrolimab failed in phase II study, pointing to additional uncovered immune checkpoints as evasion mechanisms. Using unbiased genome-wide CRISPR knockout screens in AML cell lines, we identified core-1 O-glycosylation (C1GALT1, C1GALT1C1) and mucin-like glycoprotein CD43 as dominant regulators of CD47 surface expression. Extending this screening approach across effector cell types, parallel unbiased genome-wide CRISPR screens challenging AML cells with macrophages, TCR-engineered T cells, and NK-92 cells converged on CD43 and its O-glycosylation/sialylation machinery as conserved, dominant resistance hits, establishing CD43 as a pan-immune checkpoint.Functionally, SPN/CD43 knockout, CD43-blocking antibodies, and pharmacologic O-glycosylation/sialylation inhibitors markedly enhanced macrophage phagocytosis, CD123 CAR-T/TCR-T cytotoxicity, and NK-cell killing of AML cell lines and primary patient samples. An antibody-based surface receptor screen showed that disrupting CD43 glycosylation broadly de-represses pro-immune ligands, including ICAM-1, CALR, B2M and HLA-A/B/C, central to immune synapse formation. Mechanistically, CD43 acts upstream of CD47 in a unidirectional manner, and proximity-labeling confirmed close membrane co-localization of CD43 with pro-immune ligands. We propose that the extended, rigid, heavily sialylated mucin domain of CD43 creates a steric barrier restraining effective immune cell engagement. Split-GFP reconstitution assays directly demonstrated that CD43 impairs immune cell-AML adhesion, consistent with glycan-mediated anti-adhesive shielding at the immune synapse. This function was independent of SIGLEC1/SIGLEC7 receptor signaling and membrane electrostatic charge. Strikingly, loss of pro-immune checkpoints, including ICAM-1, CALR and B2M-knockout AML cells remained fully sensitive to CD43 blockade, indicating that the O-glycan-dependent CD43 axis is distinct and non-redundant.Clinically, CD43 and core-1 O-glycosylation genes were highly upregulated in AML relative to normal hematopoietic stem cells with specific enrichment in leukemic stem/progenitor populations. Single-cell BM analyses of AML patients treated with venetoclax-azacitidine-magrolimab exhibited upregulation of SPN/CD43 expression, further supporting glycan-driven CD43 as a candidate resistance mechanism to CD47-directed therapy. Together, these findings establish glycan-dependent CD43 as a novel pan-immune checkpoint restraining innate and adaptive arms, and nominates CD43 and its glycosylation machinery as candidate therapeutic targets for AML.

(P-104) Mesangial Immune-complex Deposits of IgA Nephropathy Patients with Mesangial Hypercellularity have Reduced Exposed N-acetylgalactosamine (GalNAc) Residues of Galactose-deficient IgA1

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IgA nephropathy (IgAN) patients have circulating IgA1-containing immune complexes comprised of galactose-deficient IgA1 (Gd-IgA1), IgG autoantibodies specific for Gd-IgA1, and complement C3. Some of these circulating immune complexes accumulate in glomeruli and induce kidney injury. IgA proteins extracted from glomerular immune-complex deposits are enriched for Gd-IgA1. Our previous studies support the nephritogenic role of Gd-IgA1-IgG-C3 containing immune complexes. However, it is not known whether Gd-IgA1 molecules in the glomerular deposits have any accessible GalNAc residues that are not covered by IgG autoantibodies or complement proteins. To address this question, we stained kidney-biopsy tissues of IgAN patients with anti-IgA antibody and a GalNAc-specific lectin and used high-resolution confocal microscopy to analyze their colocalization. Colocalization results were correlated with kidney injury based on scores of the Oxford MEST-C classification. Sections of residual frozen kidney-biopsy tissues of 19 IgAN patients were stained with anti-IgA antibody and a GalNAc-specific lectin from Helix pomatia (HPA). Specificity of HPA for Gd-IgA1 was confirmed by ELISA. IgA was observed in the mesangial areas and occasionally in the glomerular capillary loops. Using confocal microscopy, 60 IgA-positive regions of interest were selected in the mesangial areas and in the glomerular capillary loops. Pearson's correlation coefficient was obtained for each region of interest to determine HPA-IgA colocalization. Mean Pearson's correlation coefficient values for each HPA-IgA pairwise colocalization in mesangial areas and glomerular capillary loops groups were correlated with Oxford MEST-C scores. Specifically, the biopsies were assigned to paired groups based on the absence or presence of each score of the Oxford MEST-C classification: M0 vs. M1, E0 vs. E1, S0 vs. S1, T0 vs. T1+T2, and C0 vs. C1+C2. HPA-IgA colocalization between mesangial and glomerular-capillary immune-complex deposits correlated (P<0.0001). Mesangial regions showed lower colocalization compared to capillary loops in all biopsies (P= 0.00036). For mesangial deposits, correlation with MEST-C scores showed lower HPA-IgA colocalization values in biopsies with mesangial hypercellularity (M1) score vs. M0 (P=0.044) consistent with fewer exposed GalNAc residues of Gd-IgA1. Colocalization in capillary loops did not correlate with MEST-C scores.This study determined that IgAN kidney biopsies have glomerular Gd-IgA1 with variably accessible GalNAc residues. Mesangial immune-complex deposits of IgAN biopsies with mesangial hypercellularity

(M1) showed less HPA-IgA colocalization,corresponding to fewer exposed GalNAc residues of Gd-IgA1. This finding may reflect Gd-IgA1-containing complexes that induce mesangial proliferative injury and contain additional molecules that restrict access to GalNAc.

(P-105) High Colocalization of MASP2 and IgA without Concurrent MBL Colocalization in Glomerular Immune-Complex Deposits of IgA Nephropathy Patients

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Most patients with IgA nephropathy (IgAN) progress to kidney failure. Glomerular immune-complex (IC) deposits contain (co)dominant IgA, variable IgG and/or IgM, and usually complement C3. Glomerular staining for mannose-binding lectin (MBL), which initiates the complement lectin pathway, and for complement C4d is associated with histologic and clinical markers of more severe disease. We used confocal microscopy to evaluate colocalization of lectin-pathway components MBL, MBL-associated serine protease (MASP) 1/3, and MASP2 with IgA in glomerular IC deposits of 28 IgAN patients. Colocalization results were correlated with histologic kidney-injury markers.Sections of remnant frozen kidney-biopsy specimens were stained with fluorochrome-labeled antibodies specific for IgA, MBL, and MASP1/3 or MASP2. In each slide, 60 regions of interest (ROIs) in the areas stained for IgA were selected in multiple optical planes obtained by confocal microscopy. Pearson's correlation coefficient colocalization data were collected for each pairwise comparison set, and the data were correlated with Oxford classification MEST-C scores and C4d immunohistochemical staining.Most samples had high Pearson's correlation coefficient colocalization values of MASP2 with IgA (mean 0.73, range 0.53-0.87), whereas no colocalization of MASP2-MBL or MBL-IgA was detected. MASP1/3-MBL showed low colocalization values (mean 0.32) and MASP1/3-IgA and MBL-IgA did not show any colocalization. Correlation analysis of MASP2-IgA colocalization with MEST-C scores using one-way ANOVA (Wilcoxon method) showed significance for sum of M+E+C greater or equal to 2 vs. less than 2 ($p=0.034$). Mixed model analysis (Least Square Means) showed significant difference for T0 vs. T1+T2 scores ($p=0.007$). Ordinal logistics calculations showed C4d correlation with M+E+C greater than 0 vs. equal to 0 ($p=0.015$) and T0 vs. T1+T2 ($p=0.024$). Our study revealed high colocalization of IgA with MASP2 without concurrent MBL-MASP2 colocalization. We propose that other lectins such as ficolin-2 and/or collectin-11 bind galactose-deficient IgA1 in immune-complex deposits and engage MASP2, activating the lectin pathway and promoting glomerular inflammation. Future analyses of these lectins will assess their role in IgAN and inform design of clinical trials targeting lectin pathway of complement.

(P-106) Glyco-phenotyping reveals glycan signature-distinct populations within the CD8+ T effector memory population

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In response to infection, T cells rapidly evolve into effector cells to fight the pathogen and following infection adapt again to form a sustained pool of memory cells that persist well after the infection resolves. T cell differentiation, activation and memory states are primarily defined using protein surface markers, termed cluster of differentiation (CD markers). While CD markers have been crucial to our understanding of T cell identity and function, this protein centric approach overlooks the dense layer of glycans that coat the T cell surface. As glycans on the cell surface of T cells have been shown to regulate T cell activation and contraction, glycosylation represents a uniquely plastic post-translational modification that presents an opportunity to further map the T cell response. Using a flow cytometry panel that incorporates lectins that recognize common glycan moieties, including poly-N-acetyllactosamine (LEL), ß-D-galactose (RCA I), complex branched N-glycans (PHA-L), α2,6-Sialic acid (SNA), and α2,3-Sialic acid (alpha 2,3-specific SiaFind), we glycophenotyped CD4+ and CD8+ T cell surface glycans over the course of influenza A/X31 infection in mice. We found that even at baseline, glycan expression differed significantly between naïve, central and effector memory CD4+ and CD8+

T cells. Notably, the composition of the glycocalyx drastically changed over the course of infection, with distinct patterns observed for each glycan tested. In particular, we found that CD8+ effector memory cells separate into two distinct subsets based on their glycan signature. While these populations could not be appreciated using CD markers alone, distinct populations marked by differential expression of polyLacNAc and -α2,6-Sialic acid were observed, one that was polyLacNAc^{high}-α2,6-Sialic acid^{low}, while the other polyLacNAc^{low}-α2,6-Sialic acid^{high}. The vast majority of antigen specific cells were found within the polyLacNAc^{high}-α2,6-Sialic acid^{low} population, suggesting that distinct glycan signatures can distinguish antigen specific responses in the absence of additional markers. These findings suggest that glycans provide a unique opportunity to characterize T cell identity beyond the use of CD markers alone.

(P-107) Comparison of autoreactive anti-ganglioside antibodies from Guillain-Barré Syndrome patients

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Guillain-Barré Syndrome (GBS) is an autoimmune disease and is the leading cause of nonpoliovirus-associated acute flaccid paralysis worldwide. Approximately 40-70% of patients develop GBS following an infection. Campylobacter jejuni gastroenteritis is the most common antecedent infection that leads to the generation of cross-reactive antibodies against C. jejuni ganglioside-mimicking lipooligosaccharides (LOS) that also recognize human gangliosides on neurons. This autoimmune reaction subsequently leads to neuropathy, from which patients can recover as their anti-ganglioside antibody titers diminish. In this study, we find 10% of recovered GBS patients still have high titers of circulating anti-ganglioside antibodies, but these antibodies cause less complement-mediated damage to human pluripotent stem cell (hPSC)-derived sensory neurons compared to antibodies isolated from the same patients during acute GBS. However, many of the serum samples from recovered GBS patients recognize C. jejuni LOS and are capable of bacterial opsonophagocytosis. Further investigation of the IgG subclasses and glycoforms indicate that the IgG glycans differ between acute and recovered GBS patients. Specifically, we isolated anti-GM1 ganglioside antibodies from recovered GBS patients and found significantly increased fucosylation compared to total IgG. It is known that IgG fucose modification decreases complement activation and decreases antibody affinity for FcγRIIIa on phagocytes. Together, these data suggest that patients with GBS may select for non-pathogenic variants of autoantibodies that are no longer capable of damaging their neurons but can still protect against C. jejuni infection.

(P-108) Desialylation of IgG Enhances Brain and Tissue Delivery in a Humanized Mouse Model

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The biochemical properties of the fragment crystallizable (Fc) portion of IgG is important for effector function, half-life, and distribution. Recent studies have demonstrated that Fc glycans can be modified after antibody secretion, suggesting that IgG residing in different tissue compartments may acquire distinct biochemical characteristics. We therefore investigated whether IgG isolated from cerebrospinal fluid (CSF) differs from matched plasma-derived IgG in people living with HIV (n = 8). These CSF-derived IgG have Fc glycosylation with a significant reduction in terminal sialic acid (p<0.05). Utilizing an in vitro a blood brain barrier (BBB) system, we found that CSF-derived IgG had increased entry compared to plasma derived IgG (p<0.05), in seven out of eight individuals . Further, we found increased entry (p<0.05) when we enzymatically removed sialic acid from Gamunex-C (pooled human IgG). We next assessed if enzymatic desialylation of IgG could lead to elevated delivery of antibody to the brain in vivo. We utilized a NSG humanized mouse model (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) with engrafted human PBMCs to evaluate the delivery of Cy5-labeled human antibody. Mice were intraperitonially (IP) injected with 3mgs of either wildtype or desialylated Gamunex-C (n=5 in each group) and at 48hrs were imaged on the in vivo imaging system (IVIS) followed by PBS perfusion and necropsy. Significantly more Cy5 signal was observed via IVIS in the brains of mice receiving desialylated gamunex-C compared to wildtype (p<0.01). Following necropsy, this finding was confirmed with brain homogenates having higher Cy5 fluorescence

in the desialylated group (p<0.01) as read on a plate reader using a standard curve derived from pre-injected labeled Gamunex-C stock. When assessing other organ homogenates, we found elevated delivery to colon and spleen (p<0.05) but not the lungs or liver. When assessing antibody levels in the blood we found that over a time course of 6hrs, 24hrs, and 48hrs, desialylated Gamunex-C exited the blood faster than wildtype with significantly less desialylated IgG in the plasma at the 48hr time point (P<0.05). Therefore, we conclude that enzymatic desialylation of IgG allows for a faster exit from the blood and an elevated accumulation in particular tissues (brain, spleen, and colon). This work has important implications for disease settings in which therapeutic antibodies need to be targeted to particular organs such as the brain and gastrointestinal tract.

(P-109) Using Multi-site Glycan Detection of Plasma IgA1 as a Readout of the Cytokine Response During Episodic Viremia in Women Living with HIV

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IgA subclass-specific glycosylation is important for inflammatory effector functions. These effector functions are dependent on sialic acid moieties, with sialic acid modification being inhibitory. IgA2 has been shown to have enhanced effector function compared to IgA1. This is primarily due to IgA1 having more extensive glycosylation which is inhibitory of effector function. IgA1 is glycosylated at N-144 and N-340 and is heavily galactosylated with close to 75% inclusion of terminal sialic acid. The relative contributions of these site specific glycosylation patterns to effector function is not well established. Therefore we set out to develop a glycan-site specific mass spectrometry readout for IgA1 glycosylation and assessed plasma IgA1 in cohort of midlife women living with HIV who experienced episodic viremia and for which cytokine and IgG glycosylation data is accessible. This cohort includes individuals from the Chicago Cook County site of the MWCCS that experienced viral loads greater than 200 copies/mL that we divided into two clusters: high viral load which is greater than 5000 copies/mL (n=6) and low viral load which is less than 1000 copies/mL (n=6). We found that IgA1 N-340 sialylation significantly correlates with IgG Fc sialylation and galactosylation (P<0.05), whereas IgA1 N-144 did not. We found N-340 sialylation to negatively correlate with IFN-Gamma in the low viral load subgroup (p<0.05), highlighting a potential anti-inflammatory role. We found N-340 sialylation to positively correlate with IL2 in the high viral load group and inversely correlates with IL-17a in the low viral group but positively correlate with IL17a in the high viral load group (p<0.05), potentially highlighting a dual role of IgA depending on the inflammatory context. When comparing between glycan sites, we found a range sialylation at both sites but only detected fucose at N-340. In this study we uncovered a differential site-specific glycosylation pattern within IgA1 that could contributes to its effector function and role in inflammation. Future work includes probing these patient-derived IgA, exhibiting a range of sialic acid levels, for their role in effector function and transport.

(P-110) ST6Gal-I modulates Treg induction and phenotype

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FoxP3+ Regulatory T cells (Treg) are a subset of CD4 T cells necessary for the maintenance of immune homeostasis, and individuals with defects in Treg populations develop early onset autoimmune disease. Treg utilize multiple mechanisms to suppress immune responses, such as anti-inflammatory cytokines, inhibitory receptors, and metabolic disruption to control or arrest activation of the innate and adaptive immune cells. Treg are highly glycosylated in mice and humans, including α2,6 sialylation. Beta-galactoside α2,6 sialyltransferase 1 (ST6Gal-I) is a broadly expressed trans Golgi enzyme that catalyzes the addition of sialic acid to terminal galactose on N-glycosylated proteins in an α2,6 conformation, and is readily expressed by Treg. We hypothesize that ST6Gal-I sialylates cell surface receptors on CD4 T cells, such as CD25, TGFβRI/II, and the T cell receptor complex, modulating Treg induction, proliferation, and maintenance. To address how ST6Gal-I regulates Treg induction, we employ in vitro cell culture to differentiate naïve CD4 T cells into induced Treg cells (iTreg) using IL-2 and TGFβ1. We demonstrate that significantly more FoxP3+ cells are induced in the absence of ST6Gal-I and this effect is dependent on TGF-β1. Next, we use mass spectrometry and Western blotting to identify the enzymatic targets of ST6Gal-I in iTreg. We find that CD25 and TGF-βRI, the receptors for IL-2 and TGFβ, are sialylated by ST6Gal-I; however, we observe no effect on downstream signaling regarding pSTAT5 and pSmad2/3, respectively. To investigate the consequences of ST6Gal-I on Treg in vivo, we analyze thymic and peripheral Treg from the spleen and colon. Analysis of splenic and colonic Treg indicate that ST6Gal-I is not required for the persistence of these populations at

homeostasis, but it supports CD25 and neuropilin-1 expression on Treg suggesting it may play a role in Treg maintenance and/or function. Paradoxically, in vivo colitis experiments reveal that ST6Gal-I diminishes the suppressive capacity of iTreg because recipients of WT iTreg had an increased Disease Activity Index and fewer FoxP3+ cells in the colon when compared to ST6Gal-I-deficient iTreg recipients. Taken together, these results show that ST6Gal-I sialylates multiple drivers of Treg induction such as CD25 and TGF-βR-1, leading to dampened induction, proliferation, and potentially maintenance of FoxP3+ CD4 T cells.

(P-111) Estrogen Receptor–Dependent Regulation of IgG Glycosylation

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Immunoglobulin G (IgG) Fc-domain glycosylation critically regulates antibody effector function, with decreased α2,6-linked sialylation consistently associated with autoimmune disease and inflammation. In contrast, IgG sialylation increases during pregnancy, coinciding with improvement of several autoimmune diseases, and rapidly declines postpartum. We have previously shown that endothelial cells (ECs) act as systemic regulators of IgG sialylation by internalizing circulating IgG and remodeling Fc glycans during neonatal Fc receptor (FcRn)-mediated recycling. However, the pregnancy-associated signals and their impact on EC-mediated IgG sialylation remain poorly understood. Estrogen (E2) is a strong candidate regulator of this process, as its levels increase dramatically during pregnancy and have previously shown to be associated with increased IgG sialylation, even outside of pregnancy. Consistent with an endothelial mechanism, our preliminary studies demonstrate that IgG sialylation progressively increases during murine pregnancy and correlates with increased ST6Gal1 expression in ECs but not B cell-derived cells. In human endothelial cells (HMEC-1), E2 appears to drive the expression of the α2,6-sialyltransferase ST6Gal1. Furthermore, genetic deletion of either estrogen receptor α (ERα) or estrogen receptor β (ERβ) in mice substantially reduces circulating IgG sialylation. Our ongoing work is focused on defining the mechanistic link between endocrine signaling via ERα and ERβ in ECs, antibody sialylation and immune quiescence.

TOPIC 12: New Emerging Concepts in Glycobiology

(KEY12-001) Dirt Is Matter Out Of Place: Glycobiology Goes Nuclear

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“Dirt,” wrote Mary Douglas, “is matter out of place.” For decades that was the verdict on any extended glycan found inside the nucleus of mammalian cells. Such findings were treated as contamination from the secretory pathway and especially the endoplasmic reticulum, whose membrane is continuous with the nuclear envelope, because the governing doctrine held that extended glycosylation belongs to the secretory pathway and the cell surface. The anomaly was not refuted so much as classified away. Here I make the case that these glycans are genuine, pervasive, and functional constituents of the nuclear proteome. In ultra-pure nuclei we detect extended, sialylated O-glycans as a common modification of nuclear proteins, with a pronounced enrichment in RNA-binding proteins. The nucleus does not synthesize them. The proteins themselves undertake a passage through the Golgi and back, acquiring their glycans from the canonical biosynthesis machinery before returning to their compartment of origin. Abolishing that return route abrogates the nuclear glycome while sparing the cell surface, revealing a dedicated trafficking circuit rather than incidental leakage. This new post-translational modification layer carries site-specific functional weight, extending to the regulation of tRNA processing and protein synthesis. What was dismissed as matter out of place turns out to have a place, and a purpose, of its own. For, where there is dirt, there is a system.

(KEY12-002) Regulated N-glycosylation controls chaperone function and receptor trafficking

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N-glycosylation, the attachment of carbohydrate chains to asparagine residues, is a post-translational modification installed on 20% of human proteins in the endoplasmic reticulum (ER). N-glycosylation plays myriad roles, including promoting the folding of membrane and secreted proteins and regulating molecular recognition events at the cell surface. All N-glycosylation is initiated by two multi-protein oligosaccharyltransferase complexes, OST-A and OST-B. Unlike enzymes like kinases or ubiquitin ligases that are tightly regulated, OST complexes are thought to constitutively catalyze glycan transfer to target sites during or after the insertion of polypeptides into the ER. Contrary to this prevailing view, we uncovered an ER pathway that modulates OST-A activity. Genetic and co-essentiality analyses linked OST-A to HSP90B1, an ER chaperone for membrane receptors, and CCCD134, an ER luminal protein we identify as the first specificity factor for N-glycosylation. Using a combination of in vitro reconstitution, mutational analysis, and structural analysis, we define a mechanism for substrate-directed regulation of OST-A activity. During its own co-translational ER insertion, an unstructured N-terminal peptide in HSP90B1 templates the assembly of a translocon complex containing CCDC134 and OST-A that protects HSP90B1 during folding, preventing its hyperglycosylation and degradation. Furthermore, disruption of this pathway impairs WNT and IGF1R signaling and causes the bone developmental disorder osteogenesis imperfecta. Thus, the major oligosaccharyltransferase complex in the ER can be regulated by ER factors to control cell surface receptor signaling and tissue development.

(PT-018) Beyond the Secretory Pathway: Oncogenic Signalling Regulates Extended Glycosylation in the Nucleus

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Extended glycosylation has long been understood as a modification of the secretory pathway and the cell surface. This view is now being reshaped by strong experimental evidence that extended glycans are also present on proteins inside the nucleus, and that this nuclear glycosylation is functionally consequential. Yet, the regulatory logic that connects cellular signalling pathways to this unexpected glycosylation compartment remains largely unknown. To address this gap, we combined genome-wide genetic screening with targeted genetic and pharmacological perturbation, using a lectin-based readout of nuclear glycosylation in human cells. A CRISPR/Cas9 knockout screen identified the Hippo/YAP pathway, a central regulator of growth and mechanotransduction that is pervasively dysregulated in cancer, as an upstream controller of nuclear glycosylation. Upstream Hippo kinases suppress the modification, while downstream YAP/TEAD transcriptional targets are required for it. Using site-directed mutagenesis, we here identify the intermediate filament protein vimentin as a key mediator, acting through a function that is independent of its canonical filament-building role. We then show that, via YAP/Vim activity, nuclear glycosylation levels follow the epithelial-to-mesenchymal cell state across multiple cell lines, placing it under the control of the same cytoskeletal and transcriptional programmes that drive invasion and metastasis in cancer. Together, these findings cement nuclear glycosylation as a signalling-responsive and disease-relevant layer of the glycome. This then frames the more broadly emerging concept that oncogenic signalling and the cytoskeleton determine not only glycan biosynthesis, but also glycan localization within the cell, with implications for how aberrant glycosylation arises and might be targeted in cancer.

(PT-019) Desmoglein 2 (DSG2) ST6GAL1 Mediated Sialylation Modulates Desmosome Ultrastructure and Strength in CRC

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Colorectal cancer (CRC) is among the most common and deadly cancers in the U.S., with a 5-year survival rate of 20% in metastatic patients. The first step of metastasis involves cell detachment, driven by disruption of cell-cell adhesive junctions, including desmosomes. Desmosome components include intracellular anchoring plaque proteins and transmembrane cadherins. Of the two cadherins, desmoglein-2 (DSG2) is currently understudied in CRC. Sialyltransferase ST6GAL1 is upregulated in various cancers, linked to poor outcomes, and known to catalyze the reaction of □2,6-linked sialic acid additions to target proteins bearing N-glycosylation sites, such as DSG2. We have previously shown sialylation can be shared between cells via ST6GAL1 transfer in extracellular vesicles (ECVs), causing treatment resistance. We hypothesize that DSG2 is sialylated via ST6GAL1 (innate or transferred) in CRC and that this sialylation alters desmosomes ultrastructure. Human SW620 CRC ST6GAL1 control (CV) and knockdown (KD) cells along with human CRC organoids were used to assess DSG2 ST6GAL1 mediated □2,6 sialylation via SNA-lectin pull-down, immunofluorescence (IF), and Western blotting (WB). Fluorescence imaging was utilized to quantify colocalization of DSG2 and Desmoplakin (DP), a desmosome specific anchoring protein. Desmosome ultrastructure and plaque to plaque distance was evaluated by Transmission Electron Microscopy (TEM) and Direct Stochastic Optical Reconstruction Microscopy (dSTORM). Desmosome function/strength was tested with a dispase cell adhesion assay. Finally, the effect of ST6GAL1 transfer on desmosome strength and DSG2 surface retention was assessed by co-culture of CV and KD cells. SNA pull-down and WB analysis of SW620 CRC cell lines and human CRC organoids revealed that DSG2 is □2,6 sialylated in CRC. The total amount of DSG2 protein was similar between SW620 CV and KD cells, as assessed by IF staining for DSG2 (CV, DSG2:DAPI = 0.03±0.01; KD, DSG2:DAPI = 0.03±0.03). The dispase assay revealed KD cells fragment less than CV cells, indicating stronger desmosomes. TEM showed that CV cells had significantly shorter desmosomes compared to KD cells. dSTORM identified that CV cells also had significantly larger gaps between DP plaques. KD cells co-cultured with CV cells showed significantly reduced strength in a dispase assay compared with KD-only cells (p=0.02). This reduced strength was similar to that observed in CV cells alone.CV cells were also found to have higher surface DSG2 than KD cells. Surface DSG2 was also increased in KD cells that were cocultured with CV cells.Based on these findings, we conclude that ST6GAL1 mediated sialylation of DSG2 in CRC, alters desmosome strength and ultrastructure. These effects can also be transferred between different cells via ST6GAL1 containing ECVs. Further study is needed to explore the impact of ST6GAL1-mediated sialylation of DSG2 in desmosome assembly as well as the effect on CRC metastasis.

(PT-020) Biological recognition of mirror image glycans

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Glycans densely coat the surface of all cells in all kingdoms of life. Chirality is critical in glycan:protein recognition. Proteins that recognize glycans bind only to a small subset of plausible stereoisomers. Prediction of stereoisomer-dependent binding is challenging for contemporary foundational ML models. Data for chirality inversion is scarce; furthermore, public data for ML training is dominated by achiral ligands. As a result, many models are not sensitive to minute changes in stereochemistry. Using in-house DNA-encoded technology termed Liquid Glycan Array (LiGA), we collect thousands of high-quality measurements for a complete set of stereoisomers of core monosaccharides across hundreds of interaction partners such as purified lectins, diverse mammalian cell types, organs inside live mice, and live bacteria. Most surprising finding was widespread binding of extant proteins to natural glycans of inverted chirality. Human and mouse immune cells show binding towards enantiomers of natural glycans, particularly L-glucose. IgMs isolated from human serum showed donor-specific recognition of L-glycans. Recognition of L-glycans by extant proteins can be due to the wide prevalence of L-glycans in the glycocalyx of many present-day microorganisms. Recognition of stereoisomers of present-day glycans by extant glycan-binding proteins expands fundamental understanding of processes such as colonization, immune response, and self-versus-nonself discrimination. This evidence-based analysis of molecular recognition makes an important contribution to the recently emerged risk assessment associated with the synthesis of enantiomeric life forms.

(P-112) Mapping Condensate Formation of Glycosaminoglycans with Cationic Peptides and Proteins

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The outer surface of the eukaryotic plasma membrane is covered by the glycocalyx, a dense, carbohydrate-rich layer on the extracellular cell surface that contains glycoproteins, glycolipids, mucins, and proteoglycans. The glycocalyx plays a protective role and contributes to membrane organization and mechanosensing. Glycosaminoglycans (GAGs), including heparan sulfate and chondroitin sulfate (CS), are major negatively charged components of this layer, and their sulfate and carboxylate groups interact with positively charged proteins and peptides. Although such electrostatic interactions are common at the cell surface, it is still uncertain if they can induce phase separation and lead to the formation of extracellular condensates. This question motivates our study of whether these electrostatic interactions can drive liquid condensates and the phase behavior varies depending on the specific GAG and its polycationic partner. Here, we study liquid condensates between heparin and CS, with the positively charged molecules poly-L-Lysine (PLL) or decaarginine (R10). Heparin is used as a model for heparan sulfate because of its similar backbone, albeit with higher sulfation levels. Microscopy data indicate that these four systems exhibit distinct phase behavior. Incubation of heparin with R10 or PLL, mostly formed coaggregation, a most solid state than condensates. In contrast, CS showed a broader range of behaviors. In the CS and R10 systems, condensate-like droplets formed at intermediate concentrations, and at higher concentrations, a mixture of liquid condensate and coaggregation droplets was observed, while no detectable droplets formed at low concentrations. CS and PLL mostly showed coaggregation. As concentration increased, the interactions showed a mixture of liquid condensate and coaggregation. These results show that the identities of macromolecules, GAGs, and polycations, and their concentrations, strongly influence phase separation. The electrostatic interactions between GAG and polycations lead to liquid condensates or coaggregation.

(P-113) Mucin-type O-glycosylation regulates epithelial morphogenesis through hemocyte-secreted Papilin and JAK/STAT signaling in Drosophila

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Mucin-type O-glycosylation is an evolutionarily conserved post-translational modification in which core 1 β1,3-galactosyltransferase 1 (C1GalT1) synthesizes T antigen, a major O-glycan structure. Loss of C1GalT1 causes developmental abnormalities in diverse organisms, but the mechanisms by which mucin-type O-glycans regulate organogenesis remain incompletely understood. Here, using Drosophila, we identify a glycan-mediated inter-tissue signaling mechanism in which embryonic hemocytes regulate epithelial morphogenesis and organ positioning.Loss of dC1GalT1 caused failure of peripodial stalk formation, resulting in mispositioning of third leg imaginal discs and subsequent adult leg malformation. Tissue-specific and stage-specific rescue experiments demonstrated that dC1GalT1 expression in embryonic hemocytes is essential for proper stalk formation. Loss of T antigen caused ectopic activation of JAK/STAT signaling in the epidermis surrounding Keilin's organ, whereas hemocyte-specific restoration of dC1GalT1 suppressed this activation. Genetic manipulation further demonstrated that an appropriate level of epidermal JAK/STAT activity is required for stalk morphogenesis.We identified Papilin (Ppn), a T-antigen-bearing extracellular matrix protein secreted by hemocytes, as a key component of this pathway. Hemocyte-specific depletion of Ppn phenocopied dC1GalT1 deficiency, causing shortened stalks and epidermal JAK/STAT hyperactivation. Biochemical analyses demonstrated that loss of dC1GalT1 markedly reduces Ppn secretion from hemocyte-derived cells.Together, these findings reveal an inter-tissue communication mechanism in which mucin-type O-glycosylation promotes secretion of hemocyte-derived Ppn, thereby regulating epidermal JAK/STAT signaling to coordinate epithelial tubulogenesis and organ positioning. Our results highlight a previously unrecognized role of glycan-modified extracellular matrix proteins in inter-tissue communication during development.

(P-114) Lipid membranes grafted with chondroitin sulfate as a platform to study the glycocalyx

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One of the defining properties of the eukaryotic plasma membrane is the glycocalyx, which is formed by glycosaminoglycans (GAGs), and glycosylated lipids and proteins. GAGs are polysaccharides composed of repeating disaccharide units that are classified depending on molecular structure and function. The glycocalyx plays a structural role that protects cell integrity, and it also participates in cell signaling, mechanosensing and receptor-like interactions. However, our understanding of its function is hampered by the lack of suitable model systems to perform biophysical investigation. To address this, we describe here the formation of lipid bilayers grafted at the outer surface with chondroitin sulfate (CS), a major glycocalyx component. CS grafting to the membrane was performed using a maleimide-thiol reaction, where a maleimide-modified phospholipid headgroup covalently binds to a thiol-modified CS. This approach was successful in the three most common types of model membranes: large unilamellar vesicles, supported lipid bilayers, and giant unilamellar vesicles. The grafting onto the membrane was confirmed via fluorescence assays, dynamic light scattering, atomic force microscopy and confocal microscopy. CS-grafted vesicles demonstrated the ability to electrostatically recruit poly-L-lysine, which in turn facilitated interactions with other CS-grafted vesicles or free CS in solution. This simple and robust method provides a versatile platform for polysaccharide grafting of lipid membranes, offering new avenues to investigate the mechanistic and pathophysiological roles of the glycocalyx.

(P-115) Cell and molecular biology of tissue-specific galectins

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Galectins are a family of at least 16 multifunctional galactoside-binding proteins with highly variable expression across cells and tissues. Most galectins are tissue-specific, whereas only four (galectin-1, -3, -8, and -9) are ubiquitously expressed at both the mRNA and protein levels. We hypothesize that tissue-specific galectins serve as essential regulators of cellular differentiation, and we provide supporting evidence for two family members: galectin-12 (LGALS12) and galectin-16 (LGALS16). First, we show that each gene is upregulated within a global galectin network in distinct human cell culture models — neutrophilic and epithelial for LGALS12, trophoblastic and neuronal for LGALS16. Second, treatment of the corresponding cell lines with recombinant galectins induces or promotes differentiation. Third, the associated signaling depends on O-GlcNAc homeostasis, redox activity, and cAMP. Finally, bioinformatic analysis of TCGA datasets shows that at least LGALS12 is downregulated in many tumors relative to normal tissues. Together, these data lead us to a broader concept in which tissue-specific galectins integrate into the global galectin network to drive cell lineage commitment, and they raise the possibility that these galectins act as tumor suppressors given their role in cell differentiation. This project has been supported by Discovery Grants from The Natural Sciences and Engineering Research Council of Canada (RGPIN-2019-06628, RGPIN-2026-05539) and the NSERC Discovery Bridge Grant from Western University (Western Research, ID 61915).

TOPIC 13: Neuroglycobiology of brain development and disease

(KEY13-001) Decoding Glycoproteome Remodeling in Alzheimer's Disease Brain

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N-glycosylation is a major post-translational modification that controls protein folding, trafficking, stability, and signaling and is therefore essential for maintaining cellular and tissue homeostasis. Although altered protein glycosylation has been implicated in Alzheimer's disease (AD), the system-level organization of the human brain glycoproteome and its disease-associated remodeling

remain poorly understood. Here, we integrated intact glycopeptide-based glycoproteomics with quantitative, network, and multiomics analyses to investigate N-glycan and glycoform alterations in the human AD brain. Our study revealed a widespread and coordinated shift in N-glycan maturation, characterized by the loss of mature, highly processed glycan structures and the accumulation of simpler and less extensively processed glycans. This remodeling included reduced sialylation, branching, and elongation, together with increased oligomannosylation and glycan truncation. Network analyses further demonstrated that these changes were organized into coordinated glycan and glycoform modules associated with AD pathology and biological processes important for neuronal function and cellular homeostasis. Alterations in components of the ER and Golgi glycosylation machinery provided additional evidence that disrupted glycan processing may contribute to the observed remodeling. Together, these findings define altered N-glycan maturation as a key molecular feature of AD and suggest that impaired glycoprotein processing may represent an underrecognized component of neurodegenerative disease biology.

(KEY13-002) O-Glycan Sialylation as a Novel Molecular Mechanism Underlying Depressive-Like Behaviors

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Major depressive disorder (MDD) is a highly heterogeneous psychiatric disorder. Clinical studies have reported altered protein glycosylation in the serum and brain of patients with depression, but whether these changes contribute causally to disease pathology remains unclear. Here, we investigated the role of mucin-type O-glycosylation in depression using glycomic, molecular, behavioral, and electrophysiological approaches. In a chronic stress-induced mouse model, we identified brain region-specific reductions in sialylated O-glycans, particularly in the medial prefrontal cortex. These changes were accompanied by decreased expression of ST3 beta-galactoside alpha-2,3-sialyltransferase 1 (ST3GAL1), a key enzyme involved in terminal O-glycan sialylation. Knockdown of St3gal1 in the medial prefrontal cortex of unstressed mice was sufficient to induce depressive-like behaviors. Conversely, restoration of St3gal1 expression in chronically stressed mice alleviated behavioral abnormalities and normalized impaired inhibitory synaptic transmission. Ketamine treatment also prevented the stress-induced reduction in St3gal1 expression. O-glycoproteomic analysis further identified altered glycosylation of neural proteins involved in synaptic organization and signaling. Together, these findings demonstrate that disruption of ST3GAL1-dependent O-glycan sialylation functionally contributes to stress-induced synaptic dysfunction and depressive-like behaviors. Our study identifies O-glycan sialylation as a novel molecular mechanism and a potential therapeutic target for MDD.

(KEY13-003) Protein O-GalNAc glycosylation in brain development and disease

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Protein glycosylation is an essential modification for brain development, but how specific glycans encode critical regulatory functions remains largely unknown. The most abundant - yet least studied - O-linked glycoproteins in the brain are of the GalNAc (or mucin) type, which are genetically associated with multiple neuropsychiatric disorders. Here, we review the unique distribution of O-GalNAc glycans primarily to the axons of neuronal fiber tracts, in contrast to N-glycans which display a more diffuse pattern in the cortex. Glycoproteomics show that O-GalNAc glycans are present primarily on critical ECM molecules including lecticans, and genetic deletion of Cosmc specifically in neurons changes the length of nodes of Ranvier, structures involved in the propagation and speed of neuronal messages. Finally, we describe more recent work highlighting roles of O-GalNAc glycans in models of congenital disorders of glycosylation, epilepsy, and neurodegeneration, with the hope that such findings will inform new diagnostic biomarkers and therapeutic targets for a range of neuropsychiatric disorders.

(KEY13-004) Glycocalyx Regulation of the Blood–Brain Barrier

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The blood–brain barrier (BBB) is formed by specialized brain endothelial cells that tightly control molecular and cellular exchange between the circulation and the central nervous system. Although BBB research has traditionally focused on endothelial junctions and transcellular transport pathways, the luminal surface of brain endothelium is coated by a dense glycocalyx composed of glycoproteins, glycolipids, and glycosaminoglycans whose functions at the BBB remain incompletely understood. Our work investigates how this endothelial glycocalyx regulates vascular permeability, endothelial signaling, and interactions between the bloodstream and the brain. Using molecular profiling, ultrastructural imaging, and targeted perturbation of specific glycan pathways, we define how glycocalyx composition contributes to BBB homeostasis and how these programs are remodeled during aging and neurological disease. We further explore whether restoring or manipulating defined endothelial glycan pathways can improve vascular function and establish the glycocalyx as a therapeutic target for preserving brain health.

(PT-021) Collaboration and antagonism between receptor protein tyrosine phosphatases and protein O-mannosyltransferases POMT1/2 in the Drosophila nervous system

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Protein O-mannosylation is an evolutionarily conserved post-translational modification essential for neuromuscular development and physiology. Canonical O-mannosylation is initiated by the obligate heteromeric protein O-mannosyltransferase 1 and 2 (POMT1/2) complex, which modifies α-Dystroglycan. Recent evidence indicates that POMT1/2 acts on a broader repertoire of functionally important substrates. We previously identified Drosophila receptor protein tyrosine phosphatase 69D (Ptp69D) as a POMT1/2 substrate required for sensory axon wiring. Our new data demonstrated that Leukocyte-Antigen-Related-like (LAR), a receptor related to Ptp69D, is also O-mannosylated in a POMT1/2-dependent manner. Despite their structural similarity and shared recognition by the POMT1/2 enzymatic complex, Ptp69D and Lar display both overlapping and divergent biological functions, and their functional relationship remains poorly understood. Previous studies reported that Lar knockdown disrupts behavior and circadian pacemaker neurons, whereas Ptp69D knockdown has no effect, revealing a distinct role for Lar in circadian regulation. Here, we investigated the interplay between Lar and Ptp69D in POMT1/2-dependent neural development. Genetic analysis of class IV sensory neuron projections in the Drosophila larval ventral nerve cord revealed distinct effects of Lar and Ptp69D disruption on axon patterning, indicating their non-redundant functions. Furthermore, manipulation of Lar or Ptp69D in POMT1/2-deficient backgrounds indicated that they have different effects on POMT1/2 phenotypes. Additionally, the analysis of Ptp69D–Lar genetic interactions revealed their antagonistic relationship, in which loss of one can partially alleviate defects associated with loss of the other. Together with our biochemical evidence demonstrating POMT1/2-dependent O-mannosylation of Lar, these findings support a model in which POMT1/2 regulate a network of functional interactions between different receptor protein tyrosine phosphatases by differentially modulating their individual functions, which tunes their concerted effect on neural development. These findings expand the functional repertoire of POMT1/2 and suggest that neurological phenotypes associated with impaired O-mannosylation may arise from altered regulation of multiple signaling proteins. Given the evolutionary conservation of these signaling proteins and the O-mannosylation pathway, our results are expected to be relevant for understanding O-mannosylation functions in the mammalian nervous system, while also shedding light on novel pathogenic mechanisms in human disorders with POMT1/2 abnormalities. This project was supported in part by the Texas A&M AgriLife BCBP Bridging Funds, A&M Targeted Proposal Teams Collaborative Seed Grant, and the AgriLife Research Capacity Support to VP, and by NSF BioFoundry: Glycoscience Research Education and Training to LW (including BioF:GREAT user project to VP).

(P-116) O-GlcNAcylation reverses Alzheimer disease molecular signatures through distinct mechanisms in males and females

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O-linked β-N-acetylglucosamine (O-GlcNAc) is a highly conserved nutrient-sensing mechanism involving the rapid attachment of a single monosaccharide, N-acetylglucosamine, to thousands of intracellular proteins. The addition of this modification is catalyzed by O-GlcNAc transferase (OGT), while its removal is catalyzed by O-GlcNAcase (OGA). Together, OGT, OGA, and the donor substrate UDP-GlcNAc regulate intracellular O-GlcNAc levels, dynamically reflecting cellular nutrient availability. In Alzheimer's disease (AD), brain O-GlcNAcylation declines as pathology advances, and pharmacological inhibition of OGA has emerged as a strategy to restore it and slow disease progression. However, whether restoring O-GlcNAc can reverse the disease associated proteome at a systems level, and whether this reversal proceeds similarly in males and females, has not been established. Here we show that the increased O-GlcNAcylation produces a significant, partial reversal of the Alzheimer's disease proteome toward the wild type state in both sexes, and that this reversal proceeded through distinct proteins in males compared with females. To test this, we performed quantitative proteomics on hippocampus from 5xFAD (AD model) and wild type mice treated with the OGA inhibitor TMG or vehicle, and analyzed males and females as fully independent cohorts. Using weighted gene co-expression network analysis, we identified a conserved module containing OGT and the insulin signaling regulators Akt1, Akt2, mTOR, and PTEN. Although compositionally identical between sexes, this module is significantly coupled to the disease genotype exclusively in females, and this pattern is independently repeated in a mitochondrial complex III and IV module. Protein interaction analysis placed OGT directly inside this insulin signaling pathway, adjacent to Foxo1, Irs1, and Pik3cb, while identifying the 20S proteasome as the central core among the proteins rescued in males. Notably, a protein's physical proximity to OGT did not dictate the extent of its reversal from the disease state in either sex, indicating that O-GlcNAcylation rescue triggers a broad systemic effect rather than a localized response spreading outward from the enzyme. Pathway enrichment and Ingenuity Pathway analysis showed that oxidative phosphorylation remained persistently elevated and unresponsive to TMG in females, whereas proteostasis and proteasome pathways were preferentially enriched in males. Furthermore, individual protein regression and global proteomic testing independently confirmed significant therapeutic reversal of AD in both sexes. However, a weak whole proteome correlation and a greater response magnitude in males indicate that TMG acts through separate molecular routes and exerts a stronger protective effect in the male brain. Altogether, these findings show that O-GlcNAcylation exerts broad systemic effects that differ fundamentally between sexes in Alzheimer's disease.

(P-117) Altered Glycosylation and Extracellular Matrix Remodeling in Severe Traumatic Brain Injury

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The brain extracellular matrix (ECM) provides structural and functional cues to facilitate cell-cell and cell-ECM communication. Blunt and penetrating brain trauma cause severe traumatic brain injuries (sTBIs), leading to widespread tissue damage, progressive ECM breakdown, and alterations in glycosylation, which have not been characterized. We hypothesize that sTBI causes aberrant glycosylation and ECM remodeling of the lesion microenvironment. We induced sTBI to the rostral forelimb area (RFA, M2 motor cortex; AP: +3.0mm, ML:+2.5mm, DV:+2.0mm) of the rat brain using a controlled cortical impact (CCI, n=3) injury, and compared it to Naive (uninjured, n=4) controls, sub-acutely, at 4-weeks post injury. Alterations in ECM and glycosylation were quantified using bulk transcriptomics, mass spectrometry-based N-glycoproteomics and N-glycomics, and immunohistochemical analyses. Multi-omic analyses at the transcriptomics and proteomics level revealed lesion specific remodeling of ECM and fibrous proteins such as chondroitin sulfate and heparan sulfate proteoglycans (CS/HSPGs), collagens, laminins, and fibronectins, post-injury (log2FC, FPKM and protein abundance, respectively). To validate findings from the transcriptomics and proteomics data, we performed histological analysis at 4-weeks post-injury. Hematoxylin and eosin (H&E) staining results demonstrated a significant perilesional upregulation in hematoxylin nucleated area in CCI animals, as compared to Naive controls. We also observed a significant upregulation in collagen deposition (Masson's Trichrome) around sTBI lesions. N-glycomic analysis revealed an overall

upregulation in oligomannose and complex/hybrid (C/H) N-glycans, and a downregulation of complex/hybrid fucosylated (C/H-F), sialylated (C/H-S), and sialofucosylated (C/H-FS) N-glycans post injury. Interestingly, Man-5 N-glycan was found to be significantly downregulated, with a significant upregulation in Man-8, and an upward trend in Man-6, Man-7 and Man-9 oligomannose N-glycans. Glycoproteomic analysis showed a similar trend in oligomannose N-glycoprotein expression, with significant downregulation of Man-5 and significant upregulation in Man-6, Man-7 and Man-9 carrying glycopeptides. Oligomannose N-glycomic and glycoproteomic expression also showed a strong correlation across both Naive ($R2 = 0.987$) and CCI ($R2 = 0.832$) groups. Taken together, these results demonstrate that sTBI induces aberrant ECM remodeling and upregulation of oligomannose N-glycosylation, indicating improper glycan trimming and extension, post-injury. These findings open new avenues for the development of advanced regenerative medicine approaches to mitigate long-term ECM and glycosylation induced tissue loss after sTBI.

(P-118) Investigating neuroglycobiology of Alzheimer's disease using human iPSC-derived neuronal model

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Human induced pluripotent stem cells (iPSCs) provide a physiologically relevant platform for modeling Alzheimer's disease (AD) by enabling the generation of cellular AD models while preserving patient-specific genetic backgrounds. Glycosylation is one of the most prevalent and complex post-translational modifications that regulate protein folding, trafficking, stability, and cell-cell communication, and is essential for neurotransmission and synaptic function. Accumulating evidence suggests that aberrant glycosylation contributes to AD pathogenesis. Although amyloid-β (Aβ) accumulation is a defining pathological hallmark of AD, how increased Aβ production reshapes the neuronal glycoproteome remains largely unknown. In this study, we performed site-specific quantitative N-glycoproteomic analysis of human iPSC-derived neurons carrying a familial AD-associated APP mutation to investigate the impact of Aβ pathology on neuronal glycosylation. Human iPSCs were differentiated into mature neurons expressing neuronal and synaptic markers including MAP2, NeuN, synaptophysin, and PSD95. We showed that APP-mutant neurons recapitulated AD phenotype and exhibited an increased Aβ42/Aβ40 ratio compared with the isogenic wild-type controls. Our analyses revealed the glycoproteomic landscape of human APP-mutant neurons and identified distinct alterations in glycoproteins and glycoforms compared to wild-type controls. These findings demonstrate that elevated Aβ is associated with remodeling of the neuronal glycoproteome. Together, our study reveals the impact of Aβ pathology on protein glycosylation in human iPSC-derived neurons and establishes a framework linking AD-associated genetic factors with neuronal glycoproteome alterations. This strategy can be readily extended to investigate other AD-related pathogenic factors, thereby advancing glycosylation-based therapeutic target discovery and biomarker development for AD.

(P-119) Dissecting the cell-type origins of GALNT2-CDG neurological deficits

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GALNT2-CDG is a rare, multisystemic disorder resulting from biallelic loss-of-function mutations in GALNT2, which encodes a GalNAc-transferase responsible for initiating mucin-type O-glycosylation. Patients with this disorder present with facial dysmorphisms and short stature as well as a broad range of neurological deficits, including global developmental delay, epilepsy, intellectual disability, and autistic-like features. Our lab generated a Galnt2 conditional knockout mouse model, and by combining this with Snap25-Cre to remove GALNT2 in post-mitotic neurons (nKO), recapitulated GALNT2-CDG patient neurological phenotypes. These findings established that neuronal GALNT2 is important for neurological function. However, neuron type-specific RNA-seq data demonstrate that GALNT isoenzymes exhibit differential expression patterns across types of neurons, and previous studies of behavioral phenotypes in mouse models of neurological disorders have related specific behavioral phenotypes to defined cell types. Therefore, we hypothesized that loss of GALNT2 in different types of neurons contributes to discrete neurological deficits observed in GALNT2-CDG. To investigate this, we generated two neuronal cell type-specific GALNT2 conditional knockout mouse models targeting excitatory and inhibitory neurons. We utilized Nex-Cre to generate glutamatergic excitatory neuron-specific knockout mice (eKO) as well as Dlx5/6-Cre to generate GABAergic inhibitory neuron-specific knockout mice (iKO). Both models underwent a comprehensive behavioral battery similar to what was carried out with pan-neuronal nKO mice,

assessing locomotor activity, anxiety-like behavior, working memory, motor coordination, and complex task integration. Behavioral characterization of eKO and iKO mice revealed overlapping and distinct impairments, with unique subsets of deficits recapitulating those observed in pan-neuronal nKO mice. Future work will identify non-redundant, isoform-specific contributions of GALNT2 to excitatory and inhibitory neuronal O-glycoproteomes. These findings provide insight into how distinct neuronal subtypes impact the neurological phenotypes associated with GALNT2-CDG, ultimately advancing our understanding of how GALNT2-mediated O-glycosylation contributes to neurological function.

(P-120) Proteomic analysis of O-GalNAc glycans in the Slc35a2-deleted cortex

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SLC35A2-CDG is an X-linked congenital disorder of glycosylation characterized by severe infantile epilepsy, developmental delay, and intellectual disability. SLC35A2 encodes the only known Golgi UDP-galactose (UDP-Gal) transporter. Disease-causing variants in SLC35A2 impair transport of UDP-Gal from the cytosol into the Golgi lumen, presumably affecting all galactosyltransferase enzymes which require UDP-Gal as a substrate. While mechanisms by which SLC35A2 deficiency leads to neurodevelopmental disorders are unclear, we have shown that this UDP-Gal transport impairment results in synthesis of truncated O-GalNAc structures on glycoproteins. However, the consequences of Slc35a2 deletion in the cortical O-glycoproteome and the protein carriers of truncated O-GalNAc structures are unknown. In this study, we utilized an Emx1-Slc35a2 conditional knock-out mouse model to selectively delete Slc35a2 in the cortex. We then used the lectin VVA to enrich and isolate O-GalNAc modified glycoproteins from the cortex, followed by liquid chromatography-mass spectrometry (LC-MS/MS) and glycoproteomic analysis. Results indicated that truncated O-GalNAc modifications primarily mapped to lecticans, a family of chondroitin-sulfate proteoglycans involved in extracellular matrix organization. Gene ontology analysis of differentially expressed glycoproteins in the Slc35a2-deleted cortex implicated biological processes involved with extracellular matrix organization and synapse biology as potentially being altered. These findings connect the genetic cause of SLC35A2-CDG with biological processes critical in normal brain development and function.

(P-121) ST6GAL1-mediated secreted glioblastoma proteins promote platelet activation

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Glioblastoma (GBM) is the most aggressive malignant brain tumor, with a median survival of only 15 months following the current standard of care. Within this highly heterogeneous tumor exists a subpopulation of tumorigenic brain tumor-initiating cells (BTICs) that drive GBM recurrence. We recently reported that BTICs exhibit elevated expression of beta-galactoside α2,6-sialyltransferase 1 (ST6GAL1), which promotes self-renewal in BTICs. ST6GAL1 is a sialyltransferase that catalyzes the addition of negatively charged sialic acid in an α2,6-linkage to select N-glycoproteins. Increased levels of ST6GAL1 and its α2,6-sialylated membrane receptor substrates have been associated with tumor progression through the activation of numerous downstream signaling pathways across multiple cancers. An unexplored area in GBM glycobiology is the interplay between secreted GBM proteins and the tumor microenvironment (TME). GBM cells secrete a wide array of proteins that are well-established mediators of multiple hallmarks of cancer. However, the secreted proteins whose levels and activity are regulated by ST6GAL1 in GBM remain largely unexplored. To address this gap, we characterized the ST6GAL1-modulated BTICs secretome using unbiased screening. Pathway analyses of the BTICs' secreted proteins identified through proteomics screening suggested that ST6GAL1 regulates extracellular matrix organization and platelet biology. Consistent with these findings, conditioned media from ST6GAL1-knockdown BTICs resulted in reduced activation of platelets from GBM patients compared with the conditioned media from non-targeting control cells. Mechanistically, galectin-3-binding protein (LGALS3BP) was identified and validated as an ST6GAL1-regulated α2,6-sialylated secreted protein. Clinically, elevated LGALS3BP levels are associated with pro-tumorigenic functions in cancer and are predictive

of thrombosis. Collectively, these findings highlight a previously unrecognized role for ST6GAL1-regulated BTICs-secreted proteins in modulating the TME through paracrine signaling.

(P-122) SLC35A2 deficiency selectively impairs O-GalNAc glycosylation in the brain of mice and humans with epilepsy

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The X-linked SLC35A2-associated congenital disorder of glycosylation (SLC35A2-CDG) causes developmental abnormalities and neurological symptoms including epilepsy and intellectual disability. However, the specific molecular alterations leading to these severe neurodevelopmental phenotypes remain unclear. SLC35A2 encodes the only known transporter for UDP-galactose (UDP-Gal), which is an essential substrate for the synthesis of all classes of glycoconjugates containing galactose, including proteins and lipids. We hypothesized that SLC35A2 deficiency would selectively impair protein O-GalNAc glycosylation in the brain given the significant overlap of MRI changes in SLC35A2-CDG and the known distribution of O-GalNAc glycans to neuronal tracts in the white matter. To determine the effect of SLC35A2 deficiency on brain glycosylation, we performed fluorescence confocal imaging and protein blotting using a panel of lectins on brain tissue from brain-specific Slc35a2-deficient mouse model and human brain biopsies from cases of SLC35A2-associated intractable epilepsy. In Slc35a2 deficient mouse brain, O-GalNAc glycans detected by PNA did not properly localize to neuronal tracts, while truncated O-GalNAc glycans detected by VVA accumulated in the cortex. Several N-glycan binding lectins had no change in their distribution. We confirmed this finding with protein blots, finding a significant reduction in PNA binding and a subsequent build-up of VVA in the Slc35a2-deficient cortex, while binding of several N-glycan binding lectins were unchanged, except GSL-II. Human brain biopsy samples with SLC35A2 mutations showed the same selective glycosylation differences, with significant changes in PNA and VVA but no N-glycan binding lectins, including GSL-II. These findings suggest that O-GalNAc glycosylation is more vulnerable than other pathways in the brain and may be the primary mechanism whereby SLC35A2 deficiency causes epilepsy.

(P-123) Distinct Roles of STT3A and STT3B in Maintenance of ER and Golgi Morphology and Homeostasis

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N-linked glycosylation is a common protein modification that occurs both co-translationally and post-translationally. STT3A and STT3B are the catalytic subunits of oligosaccharyltransferase (OST) complex A and B, respectively, which transfer oligosaccharides to specific asparagine residues of substrate proteins. The STT3A complex catalyzes co-translational N-glycosylation of nascent polypeptides, whereas the STT3B complex predominantly mediates post-translational N-glycosylation of sites skipped during protein synthesis. Genetic defects in either STT3A or STT3B cause congenital disorders of glycosylation (CDGs) with neurological abnormalities. Furthermore, studies from our group and others have shown that both STT3A and STT3B expression, as well as global N-glycosylation, are altered in the brains of patients with Alzheimer's disease. However, the molecular mechanisms underlying these alterations and their functional consequences remain to be clarified. To investigate these mechanisms, we used CRISPR/Cas9 gene editing to disrupt STT3A or STT3B expression in SH-SY5Y human neuroblastoma cells, a widely used neuronal cell model. Our analyses demonstrate that loss of either STT3A or STT3B differentially alters the abundance of high-mannose, hybrid, and complex N-glycans in whole-cell lysates as well as in isolated ER and Golgi fractions. We found that knockout of STT3A or STT3B induces pronounced but distinct morphological alternations in both the ER and Golgi apparatus. In addition, Western blot analyses revealed differential regulation of ER stress markers following STT3A or STT3B knockout, indicating that the two OST catalytic subunits exert distinct effects on ER homeostasis. Collectively, our findings reveal distinct roles of STT3A- and STT3B-mediated N-glycosylation in maintaining ER and Golgi integrity and provide new insights into the mechanisms by which aberrant N-glycosylation contributes to neuronal dysfunction and the pathogenesis of CDGs and Alzheimer's disease.

TOPIC 14: Systems and Integrative Glycobiology

(KEY14-001) Federated Data, a National Glycome Atlas, and Agentic AI: Layers of Infrastructure for Systems Glycobiology

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Systems-level understanding of glycobiology depends on the ability to connect glycan structures, glycosyltransferases, glycoproteins, glycogenes, and disease associations that are otherwise scattered across independently maintained databases. GlyCosmos Portal (glycosmos.org) addresses this fragmentation by representing heterogeneous glycoscience data as RDF triples following the GlycoRDF ontology and WURCS glycan notation, cross-linked to major bioresources such as UniProt, ChEBI, PDB, Reactome, and GlyGen through shared identifiers and SPARQL-queryable linked data. This talk briefly summarizes this integration strategy and the standards it relies on.We then turn to how this infrastructure is being scaled for systems glycobiology through the Human Glycome Atlas (HGA) project, a MEXT-funded Large-scale Academic Frontiers Promotion Project (with the Tokai National Higher Education and Research System and the National Institutes of Natural Sciences) that aims to establish the first comprehensive, standardized reference for the human glycome. HGA is generating high-throughput glycomic, glycoproteomic, and glycogenomic data from a planned cohort of 20,000 blood samples, integrated with clinical and disease metadata. We are developing TOHSA (Total Human Saccharide Atlas), the knowledgebase that will serve as the central access point for this resource, built on the same RDF and ontology foundations as GlyCosmos so that population-scale human glycome data can be linked directly to existing glycan, protein, and pathway knowledge. We discuss the data model, integration challenges specific to large-scale human cohort data, and our roadmap toward an access-controlled public release.Finally, we present early work, led by an undergraduate researcher in our group, on making these integrated resources queryable by AI agents via a Model Context Protocol (MCP) server. The server translates natural-language questions about glycan structures, biosynthetic pathways, and disease or cohort associations into SPARQL queries against GlyCosmos, and prospectively TOHSA, returning results an LLM-based agent can reason over and present conversationally. We discuss design choices for mapping an ontology-driven RDF schema to reliable agent tool calls, early use cases, and open challenges in query accuracy and evaluation.Together, GlyCosmos, HGA/TOHSA, and this emerging agent interface trace a path from federated public data integration to population-scale systems glycomics to machine-actionable access, infrastructure intended to make integrated glycoscience data usable by both human researchers and the next generation of AI-assisted research tools.

(KEY14-002) From Systems-Level Glycan Pathway Analysis to Cellular Engineering of Human Milk Oligosaccharide Biosynthesis

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Glycosylation is governed by coordinated networks of glycosyltransferases, glycosidases, nucleotide-sugar transporters, and other related factors. However, translating gene expression data into an understanding of cellular glycan biosynthetic capacity remains challenging. To address this issue, we developed GlycoMaple, a web-based platform that maps transcriptomic data onto glycan biosynthetic pathways and predicts the potential of cells and tissues to generate specific glycan structures. GlycoMaple provides a systems-level framework for identifying key enzymatic steps that may limit or redirect glycan production. We applied this concept to the biosynthesis of human milk oligosaccharides (HMOs), which are normally produced in mammary epithelial cells but rarely in commonly used cultured mammalian cells. Pathway analysis suggested that lactose production is a critical entry point for reconstructing HMO biosynthesis. Expression of α-lactalbumin (LALBA) enabled HEK293 cells to produce multiple HMOs, including sialylated and fucosylated structures. Further manipulation of glycosyltransferase genes altered the abundance and structural diversity of the products. The engineered HMOs also promoted the growth of Bifidobacterium, demonstrating their biological activity. These findings illustrate how systems-level analysis of glycan biosynthetic pathways can guide targeted glycoengineering. The integration of GlycoMaple with cellular engineering provides a general strategy for predicting, reconstructing, and tailoring complex glycan biosynthetic pathways.

(PT-022) Glycogene-Specific Polygenic Risk Score Predicts Glycemia in Type 2 Diabetes And Is Modified By Nutrient Exposure

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Type 2 diabetes (T2D) risk reflects interactions between genetic susceptibility and environmental exposures, yet glycosylation-related genetic risk has rarely been examined in this context. We developed a glycogene-specific polygenic risk score (PRS) for T2D using genome-wide association study summary statistics (n=60,975; 39% T2D cases) and genome-wide genotype data from the Omics in Latinos study (Ola, n=11,720; 20% T2D cases), and independently validated in a smaller cohort from the El Banco Biobank (n = 886, 61% cases). The PRS was constructed using LDpred2 with a 70/30 train/test split. Dietary intake was assessed using validated multi-pass 24-hour recalls with at least two recalls per participant. Associations between the PRS and T2D risk were evaluated using multivariable logistic regression adjusting for demographic, study-center, and ancestry covariates. PRS×nutrient interactions were evaluated using multivariable-adjusted models and repeated discovery–holdout analyses of dietary principal components.In the Ola study, glycogene-specific PRS was associated with higher T2D risk (OR: 1.23, p< 0.0001), showed modest standalone discrimination (AUC= 0.56), which improved when age and sex were added to the model (AUC = 0.75). In the independent validation set, the association was directionally consistent but not statistically significant (OR=1.11, p=0.154; AUC=0.52). Across both datasets, PRS was primarily driven by SNPs within ST6GAL1, NDST4, B3GALT1, FUT8 and CHST11, implicating N-glycosylation (sialylation, galactosylation and fucosylation) and proteoglycan (heparan and chondroitin sulfate) biosynthesis pathways. The manifestation of glycogene risk (ie PRS) into glycemic outcomes (fasting glucose, HbA1c) were moderated by nutrient exposure. In the Ola study, for individuals with higher glycogene PRS, a nutrient intake profile with lower long-chain polyunsaturated fatty acids, including EPA and DHA, and higher B-vitamins (B2, B3, B12, Folate) was associated with lower fasting glucose ($p = 0.004$, $q = 0.009$). These findings support a systems-approach to understand glycosylation-related genetic susceptibility with complex environmental exposures and suggest that the expression of glycogene-associated T2D risk may vary depending on nutrient exposure. Ongoing work is focused on identifying SNP × nutrient interactions to further narrow down specific molecular pathways of interactions.

(PT-023) Gastric epithelial differentiation shapes mucin expression, glycosylation and mucus properties in human organoid monolayers

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The gastric epithelium contains distinct mucin-producing cell populations that contribute to the composition and properties of the mucus barrier. However, how epithelial differentiation shapes mucin expression, glycosylation and mucus properties remains difficult to study in human experimental models. We previously profiled gastric, jejunal and colonic 3D PDOs using glycomics, proteomics and lectin-based analyses. The models retained regional mucin expression and O-glycan features, with gastric PDOs showing shorter, more fucosylated glycans and intestinal PDOs showing increased sialylation and more elongated core-2 structures. Lectin profiling supported these regional trends. However, mature intestinal core-3/core-4 glycosylation was poorly represented, while matrigel-derived N-glycans substantially confounded epithelial N-glycomic analysis.To address these limitations, we established matrigel-free gastric PDO monolayers with accessible apical and basolateral compartments, enabling direct analysis of mucin secretion under gland- and pit-directed differentiation conditions. Quantitative proteomics separated the two states, with gland-type cultures enriched in MUC6, TFF2, A4GNT and PGC, and pit-type cultures enriched in MUC5AC, TFF1, GKN1/2, PSCA and CEACAM5. Differentiation was accompanied by changes in glycosylation machinery, including increased B3GNT6/B3GNT7 in pit-type cultures. Preliminary O-glycomics supported these molecular differences, with relatively higher core 1/2 O-glycans in gland-type cultures and increased core 3 structures in pit-type cultures. The monolayer system further enabled direct characterization of the secreted mucus layer, with scanning electron microscopy revealing mucin ultrastructure and FRAP assessing mucus

mobility.Together, these findings establish a gastric monolayer model that integrates epithelial differentiation with mucin expression, glycosylation and mucus properties, with potential applications in gastric disease modelling.

(P-124) Site-Specific Intact N-Glycoproteomics Reveals Multi-Level N-Glycosylation Remodeling during the Monocyte-to-Macrophage Differentiation

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Monocyte-to-macrophage differentiation is critical for human immune systems, and during the differentiation, the properties and functions of cells change dramatically. Protein N-glycosylation plays extremely important roles in regulating cell immune response. However, glycan profiling and deglycosylation-based N-glycoproteomics measure either the bulk glycome separated from glycoproteins or the relative abundance of formerly N-glycosylated peptides after glycan removal, but they cannot link specific glycans to the individual glycosylation sites that carry them. Here, we systematically studied intact N-glycoproteome changes during the differentiation from monocytes to macrophages by integrating intact glycopeptide enrichment with multiplexed proteomics. We identified 76,048 intact N-glycopeptides spanning 4,454 N-glycosylation sites on 1,720 glycoproteins, and quantified differentiation-associated remodeling at multiple levels, from glycan, glycoform, glycosylation site, to glycoprotein. The microheterogeneity of glycosylation sites varied among glycoproteins in different cellular compartments, and was higher on post-ER glycoproteins than on ER-resident ones. During the differentiation, the results revealed that a substantial fraction of the N-glycosylation changes was independent of their corresponding parent-protein abundance alterations. At the site level, the changes mainly occurred in receptor families involved in adhesion, recognition, and signaling. Furthermore, glycoproteins associated with phagocytosis and efferocytosis were more abundant in macrophages than in monocytes, consistent with the phagocytic and efferocytotic functions acquired during the differentiation. The high-mannose fraction also increased at specific sites on up-regulated clearance receptors, including the macrophage marker CD163. This work provides a comprehensive and site-specific resource of protein N-glycosylation changes with intact glycan information during the monocyte-to-macrophage differentiation. The results offer deeper insight into how glycosylation helps establish macrophage identity and function and contribute to a better understanding of glycoprotein functions.

(P-125) Comprehensive Characterization of Glycoproteins on the Cell Surface and Their Dynamics using Mass Spectrometry-Based Proteomics

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The surface of mammalian cells contains many important proteins, and nearly all of them are glycosylated. These surface glycoproteins play extremely important roles in regulating many extracellular events from cell-cell communication, cell-matrix interactions, and cell immune response. Aberrant protein glycosylation on the cell surface is directly related to human diseases, such as cancer and infectious diseases. Comprehensive analysis of surface glycoproteins can allow us to identify biomarkers for disease detection and drug targets for disease treatment. We have worked on the development of innovative and effective mass spectrometry (MS)-based chemical and enzymatic methods to globally and site-specifically analyze glycoproteins only located on the cell surface. Integrating metabolic labeling and bioorthogonal chemistry, we specifically tagged surface glycoproteins for their selective enrichment [1]. In combination with MS-based proteomics, we performed comprehensive and site-specific analysis of surface glycoproteins. Moreover, we developed enzymatic methods to specifically tag surface glycoproteins for their analysis by MS. One main advantage of these methods is that the conditions are mild, enabling us to study the dynamics of surface glycoproteins [2-4]. Cell-surface glycoproteins are highly dynamic for cells to adapt to everchanging environment. We systematically quantified the dynamics of surface glycoproteins in monocytes and macrophages in response to the infection, and novel surface glycoproteins related to the infection were identified [3]. Furthermore, we performed systematic analysis of surface glycoproteins in more physiologically relevant primary human macrophages with M1 or M2 phenotype (HM1 or HM2), and the results reveal extensive, phenotype-specific remodeling of the cell-surface glycoproteome: HM1 display enhanced innate immune signaling, whereas surface glycoproteins related to adhesion and endocytosis are upregulated in HM2. Integrative analyses of cell-surface glycoproteomic, transcriptomic, and tissue-specific datasets helped identify surface proteins as drug targets to inhibit M2 macrophages, including LIPA, CD200R1, and CR1. Orthogonal immunofluorescence imaging further validated phenotype-specific abundance changes of

several key surface glycoproteins [5]. Considering the importance of surface proteins, the methods will have extensive applications in biology and biomedicine, which will result in a better understanding of glycoprotein functions, cellular activities, and disease mechanisms.References:1. W. Chen, J. Smeekens, R. Wu*, Chem. Sci. 6(8): 4681, 2015.2. H. Xiao, R. Wu*, Chem. Sci., 8(1): 268, 2017. 3. S. Suttapitugsakul, M. Tong, R. Wu*, Angew. Chem. Int. Ed., 133: 11595, 2021.4. X. Xu, K. Yin, R. Wu*, Mol. & Cell. Proteomics 23 (5): 100761, 2024. 5. X. Xu, Y. Wu, K. Yin, R. Wu*, Nat. Commun., accepted, 2026.

TOPIC 15: Proteoglycans, Glycosaminoglycans, and ECM Biology

(KEY15-001) Age-associated disruption of the hyaluronan matrix impairs cumulus-oocyte complex expansion and ovulation

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Ovulation is the process by which a mature egg within a cumulus-oocyte complex (COC) is released from the ovary for fertilization. As part of ovulation, the cumulus cells undergo expansion through the production of a extracellular matrix that is essential for follicle rupture and successful fertilization. One major ECM component is hyaluronan (HA), a glycosaminoglycan synthesized primarily by hyaluronan synthase 2 (HAS2). In response to the ovulatory signal, production of HA and its associated proteins, PTX3 and TNFAIP6, is substantially increased, while defects in this process lead to subfertility and infertility in mouse models. We have previously shown that pharmacological and physiological disruption of the HA matrix leads to reduced cumulus expansion and delayed ovulation. We demonstrated that treatment of COCs with a proprotein convertase inhibitor (PCI) during ovulation decreases expression of HAS2, PTX3, and TNFAIP6, leading to dysregulated HA distribution and subsequent loss of COC matrix integrity. This loss of matrix integrity translated to premature cumulus cell dispersion and reduced follicle rupture in vitro. We have also demonstrated that reproductive aging causes substantial defects in HA production and COC matrix integrity during ovulation. We found that COCs from reproductively old mice have reduced cumulus expansion and COC area compared to those from young mice. Additionally, reproductively old COCs exhibited reduced HA levels and expression of HA-associated proteins throughout the ovulation window. We observed that the extracellular matrix integrity of expanded COCs was compromised with advanced age as evidenced by increased penetration of fluorescent nanoparticles in a particle exclusion assay and larger open spaces on scanning electron microscopy. These findings translated to ovulatory kinetics in vivo, where reproductively old mice exhibited an increased proportion of unruptured follicles and delayed ovulation compared to young mice. Overall, our work indicates HA is a critical component of the COC extracellular matrix that is essential for cumulus cell expansion, follicle rupture, and successful ovulation. These findings are essential in understanding whether disruptions in matrix integrity are associated with clinical infertility and the opportunity for HA-based therapeutics to improve fertility outcomes.This study was supported by the Eunice Kennedy Shriver National Institutes of Child Health and Development (R01 HD093726, M.T.P. and F.E.D.; K12 HD050121, E.B.; T32HD094699, C.E.K.) and the Gates Foundation (INV-003385, INV-084649, F.E.D.).

(PT-024) Intact and single-molecule analysis of heparan sulfate

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Establishing tools to couple biological processes to a DNA sequence has transformed our ability to monitor life at the molecular scale due to the scalability, flexibility, and low cost of DNA sequencing. Key examples include DNA-protein (ChIP-seq), RNA-protein (CLIP-seq), protein-protein (proximity ligation assay), and Cas-based recording of cellular events. In contrast, this paradigm has not yet significantly enhanced studies of glycans, which are mostly limited to non-DNA based chemical and biochemical assays. While classical asparagine-linked and serine/threonine-linked glycans can be directly sequenced using mass spectrometry, glycosaminoglycans - key scaffolds of the extracellular matrix - cannot be easily analyzed in their full-length form. Here we introduce HS-nano-seq, a generalized framework to selectively label, process, and detect features of heparan sulfate on a nanopore sequencing platform. Recognizing that heparan sulfate is biochemically analogous to a nucleic acid, we report purification techniques using rapid nucleic acid strategies and conjugation methods to couple DNA adapters, generating HS-DNA chimeras resolved as discrete species by capillary electrophoresis (CE). The CE assay can distinguish features of chain length and sulfation patterns. At the single-molecule level enabled by nanopore sensing, we classify a library of synthetic heparan sulfate standards and demonstrate that nanopore ionic current fingerprints encode sulfation-dependent structural features of individual HS chains. Analysis of intact, cell-derived HS could discriminate features of individual chains with different sulfation patterns, defining the heterogeneity of binding motifs across cell types and how cells organize and program the tethered extracellular matrix. More broadly, HS-nano-seq establishes a framework for achieving full-length readouts of ECM glycopolymers that are amenable to the same biological interrogation as nucleic acids.

(PT-025) A non-canonical EZH2/TRIM28 epigenetic axis drives heparan sulfate remodeling and melanoma metastasis

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Melanoma progression and metastasis are driven not only by oncogenic alterations but also by epigenetic programs that dynamically remodel the tumor microenvironment. Heparan sulfate (HS) proteoglycans are key extracellular matrix components that integrate growth factor signaling, cell-matrix interactions, and migratory behavior by controlling ligand availability and receptor engagement, yet how chromatin-associated factors regulate HS remodeling in cancer remains poorly defined. Here, we identify the histone methyltransferase EZH2 as a key regulator of HS biosynthesis in melanoma. Integrated bioinformatic and genomic analyses revealed enrichment of EZH2 and additional Polycomb Repressive Complex (PRC) factors at regulatory regions of HS biosynthetic genes. CRISPR-mediated loss of EZH2 altered expression of multiple HS-modifying enzymes, most notably the secreted endosulfatases SULF1 and SULF2, resulting in enhanced HS 6-O sulfation and altered ligand binding at the cell surface. Unexpectedly, EZH2 promoted SULF1 expression through a methyltransferase-independent mechanism via a non-canonical interaction with TRIM28, whereas SULF2 was regulated through canonical PRC2-mediated repression. Functionally, SULF1 depletion impaired melanoma cell migration and invasion in vitro and reduced spontaneous metastasis in an orthotopic xenograft model. Together, these findings define an epigenetic axis linking chromatin regulation to extracellular glycan remodeling and identify HS-modifying enzymes as candidate targets to limit melanoma metastasis.

(P-126) Hypothalamic perineuronal nets undergo biphasic remodeling in response to high fat diet

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The hypothalamic arcuate nucleus (ARC) contains glucose-responsive neurons, including AgRP/NPY populations, enmeshed by perineuronal nets (PNNs) that are comprised of the core protein proteoglycan, aggrecan (ACAN). Attached to ACAN are chondroitin sulfate-glycosaminoglycan (CS-GAG) chains that are detected by Wisteria floribunda agglutinin (WFA) lectin labeling. Although prior reports suggest that ARC PNN abundance increases with short-term high-fat diet (HFD) feeding and contributes to obesity-associated metabolic impairment, the effects of chronic HFD intervention have not been investigated. Here, we performed a thorough time course characterizing the changes in ARC PNN abundance in mice fed HFD (60% kcal fat, 20% kcal carbohydrates) or high-fat, high-sucrose (HFHS, 58.8% kcal fat, 26.3% kcal carbohydrates) diets from 3 days to 12 months. We found that WFA+ ARC PNN abundance was unchanged at 3 days ($p=0.83$) but significantly increased by 7 days ($p=0.04$) and 28 days ($p=0.04$) of HFD. Interesting, WFA+ ARC PNN abundance returned to baseline by 3 months of HFD, where it then significantly declined by 4.5 months (HFD $p=0.0037$; HFHS $p=0.029$) and 12 months ($p=0.016$) of dietary exposure. Importantly, this biphasic response in ARC PNN abundance followed peripheral blood glucose expression, thus tightly correlating changes in ARC PNNs with peripheral metabolic dysregulation.This biphasic response was also reproduced across both HFD and HFHS interventions and was absent in retrosplenial granular cortex, indicating obesity-driven, ARC-specific remodeling. In contrast, (db/db) mice, which are hyperphagic and obese on chow diet, exhibit elevated ARC PNNs even in the absence of HFD compared to wild-type chow controls ($p=0.0007$) that was not further elevated by 28 days of HFD ($p=0.93$), suggesting that obesity itself is sufficient to increase ARC PNNs. Using a 3-4h ChondroitinaseABC pre-digestion protocol on both chow and HFD-fed mouse brain sections, which reduced WFA+ CS-GAG labeling by 68.6-96.3% and increased ACAN+ PNN detectability 1.7-1.8-fold, we found ACAN+ PNN abundance did not change from 3 days through 3 months, when WFA+ PNNs were elevated, but significantly declined at 4.5 months (HFD $p=0.0006$; HFHS $p=0.021$) and a trend for reduction at 12 months ($p=0.08$) of HFD, despite sustained WFA+ ARC PNN decline. These findings indicate that the early-phase increase in ARC PNNs may be a CS-GAG-specific event occurring without ACAN core protein change, whereas the second-phase decline involves loss of both CS-GAGs and ACAN itself. Together, these results establish ARC PNNs as highly plastic, compositionally distinct across phases of DIO that may influence acute and chronic changes in peripheral blood glucose.

(P-127) Syndecan-1 Controls Hepcidin Expression and Iron Homeostasis Through BMP-SMAD Signaling in the Liver

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Systemic iron homeostasis is essential for supporting metabolic function and preventing iron toxicity. The liver-derived peptide hormone hepcidin plays a central role in this process by promoting degradation of ferroportin, the only known mammalian iron exporter. Hepcidin expression is tightly regulated by iron status, inflammation, and erythropoietic demand, primarily through the BMP/SMAD and IL6/STAT3 signaling pathways. Although heparan sulfate (HS) has been implicated in both BMP and inflammatory signaling, the specific HS proteoglycans that govern hepcidin regulation have remained undefined.Here, we identify Syndecan-1 (SDC-1) as a key regulator of hepatic hepcidin expression. Genetic and pharmacologic disruption of SDC-1 in human hepatoma cells and primary hepatocytes markedly reduced both basal and BMP6-induced hepcidin transcription. Mechanistically, SDC-1 functions as a co-receptor to support BMP/SMAD signaling, enabling efficient SMAD1/5/8 phosphorylation and activation of the HAMP promoter. Notably, SDC-1 shedding attenuated BMP-driven hepcidin induction, consistent with a requirement for membrane-bound SDC-1 in signal transduction. In contrast, the addition of shed SDC-1 was sufficient to rescue hepcidin expression in SDC1-deficient hepatocytes. Consistent with these findings, hepatocyte-specific deletion of SDC1 in vivo suppressed hepatic hepcidin expression. In contrast, SDC-1 deficiency did not affect hepcidin induction or iron handling in response to inflammatory stimuli such as IL-6, indicating that SDC-1 is dispensable for the inflammatory control of iron metabolism.These findings establish Syndecan-1 as a key structural and functional regulator of hepcidin and iron homeostasis. By bridging extracellular matrix components with intracellular signaling, SDC-1 serves as a gatekeeper of hepatic iron sensing. Its loss disrupts iron balance and contributes to disease states characterized by iron overload or inflammatory anemia. Targeting the SDC-1–BMP-hepcidin axis may offer novel therapeutic strategies for the treatment of iron-loading disorders and chronic inflammatory diseases associated with dysregulated iron metabolism.

(P-128) Biochemical characterization of an atypical variant affecting the initiation codon in CHST14 in a patient with musculocontractural Ehlers-Danlos syndrome

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[Introduction] Musculocontractural Ehlers-Danlos syndrome (mcEDS) caused by pathogenic variants in the carbohydrate sulfotransferase 14 gene (CHST14) (mcEDS-CHST14) is an autosomal recessive connective tissue disorder characterized by congenital malformations including specific craniofacial features, congenital multiple contractures, and progressive fragility-related complications. CHST14 encodes dermatan 4-O-sulfotransferase-1 (D4ST1), which is responsible for the biosynthesis of dermatan sulfate (DS). DS plays a number of roles in a wide range of biological activities such as cell signaling and tissue morphogenesis through interactions with various growth factors and extracellular matrix proteins containing collagens. A nonsense variant and four missense variants of CHST14 in patients with mcEDS-CHST14 were found to show a decreased sulfotransferase activity [Miyake, Kosho, and Mizumoto et al., Hum. Mutat. 31, 966-974, 2010]. However, it remains unclear how an atypical pathogenic variant in the initiation codon of CHST14 affects its translation, enzymatic activity, and subcellular localization. To characterize the atypical variant, a 9-bp deletion affecting the initiation methionine codon in CHST14 (c.2_10del: p.M1_R4del), and glycobiological analyses were conducted.

[Methods] Sulfotransferase activity from the recombinant D4ST1 and cultured skin fibroblasts of the patient, and cellular localization of the mutant D4ST1 were examined. The levels of DS from urine and fibroblasts of the patient were quantified by combination of treatment with chondroitinase B and anion-exchange chromatography.

[Results & Discussion] The mutant enzyme showed a lower molecular weight (~10 kDa) and a considerably decrease in sulfotransferase activity compared with the wild-type, suggesting that another initiation codon in the coding region of CHST14 might function during translation, and that the mutant D4ST1 reduces the enzymatic activity. Furthermore, the wild-type D4ST1 was localized in Golgi apparatus, whereas mutant D4ST1 was observed in the cytoplasm and nucleus. DS was not detected in the fibroblasts as well as urine from the patient. These findings indicate that the truncated D4ST1 protein was estimated to exert a complete loss of function due to the intracellular mislocalization and a reduction in sulfotransferase activity, which resulted in mcEDS-CHST14. This is the first study to characterize a variant affecting the initiation codon of CHST14 in mcEDS-CHST14 glycobiologically.

(P-129) Regional Distribution of Heparan Sulfate Across the Porcine Sclera: A Pilot Immunofluorescence Study

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Background: The sclera is a collagen-rich connective tissue whose microstructure and mechanical properties vary regionally, influencing ocular biomechanics and potentially contributing to myopia and glaucoma. Glycosaminoglycans (GAGs) modulate extracellular matrix organization in collagen-rich tissues and may contribute to these regional differences. However, the regional distribution of individual GAG classes across the sclera remains incompletely characterized.Purpose:This pilot study examined the regional distribution of heparan sulfate (HS), one of the major GAG classes, across the anterior, equatorial, and posterior porcine sclera. Methods: Scleral tissues from the anterior, equatorial, and posterior regions of a single porcine eye were fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose, embedded in optimal cutting temperature compound, and cryosectioned at 5 µm. Sections were treated with cold acetone and stained for HS using an immunofluorescence protocol adapted from Clark et al. (2011), with the 10E4 anti-HS primary antibody (1:20), Alexa Fluor 488-conjugated secondary antibody (1:200), and DAPI counterstaining. Secondary-only controls assessed nonspecific secondary antibody signal. HS staining specificity was evaluated by pretreating separate sections with heparinases I, II, and III (20 U/mL each) for 1 h at 37°C before immunostaining. Images were acquired using a Leica SP8 confocal microscope with consistent acquisition settings. For preliminary quantitative analysis, one 488-channel image from one tissue section per region was analyzed in Fiji/ImageJ using a mean fluorescence intensity (MFI) ROI approach adapted from Shihan et al. (2021). Background-corrected MFI was calculated by subtracting the mean intensity of three non-tissue background ROIs from the tissue MFI. Results: Strong HS staining was detected in all three scleral regions. Secondary-only controls showed no detectable fluorescence. Heparinase treatment eliminated detectable HS fluorescence in the anterior and equatorial sclera

but only reduced the posterior signal. Background-corrected MFI differed across the analyzed images: 18.13 a.u. anteriorly, 12.04 a.u. equatorially, and 36.50 a.u. posteriorly. Conclusions: The adapted immunofluorescence approach enabled detection and regional assessment of HS in porcine sclera. Preliminary analysis showed regional variation in HS fluorescence, with the highest signal observed in the posterior sclera. Studies with additional eyes and sections are needed to confirm this pattern. Future work will extend immunofluorescence to the other major GAG classes and use liquid chromatography-tandem mass spectrometry (LC-MS/MS) for direct quantitative characterization of scleral GAG composition.

(P-130) Hypoxia suppresses vascular endothelial proteoglycan secretion by disrupting the balance of ER-to-Golgi intracellular trafficking

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As atherosclerotic lesions progress, arterial wall thickening leads to the formation of a localized hypoxic microenvironment. Because endothelial cells are directly exposed to the bloodstream, they are among the first vascular cells affected by hypoxia. However, the effects of hypoxia on endothelial cell function remain incompletely understood. Endothelial proteoglycans, including perlecan, syndecan-1, and biglycan, are major components of the endothelial glycocalyx, which regulates vascular barrier function and signaling. Hypoxia (1% O2) upregulated perlecan and biglycan mRNA expression in bovine aortic endothelial cells via HIF-1α, whereas syndecan-1 mRNA showed no significant change. In contrast, core protein levels of all three proteoglycans were markedly reduced. This reduction could not be explained by HIF-1α activity, decreased translational efficiency, or extracellular degradation by matrix metalloproteinases. These findings suggest that the decrease results from impaired post-translational intracellular trafficking and secretion. Treatment with brefeldin A, an endoplasmic reticulum (ER)-to-Golgi transport inhibitor, suppressed core protein secretion, confirming that proteoglycan secretion relies on the ER-to-Golgi pathway. Furthermore, hypoxia decreased the activity of SAR1, a mediator of anterograde ER-to-Golgi transport, while increasing the activity of ARF1, which mediates retrograde Golgi-to-ER transport, thereby disrupting intracellular trafficking balance. Consistent with these transport alterations, immunofluorescence staining revealed reduced localization of perlecan and syndecan-1 to the Golgi apparatus under hypoxia. Taken together, these findings suggest that impaired ER-to-Golgi transport suppresses proteoglycan secretion, representing a previously unrecognized mechanism that may contribute to vascular dysfunction in atherosclerotic lesions.

(P-131) Programmable heparan sulfate mimetic engagement of G6b-B drives thrombopoiesis

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De novo platelet production is insufficient to meet physiologic demand during thrombocytopenia, prompting the development of strategies to enhance thrombopoiesis. Over 2.2 million platelet transfusions are performed annually in the U.S. to ameliorate thrombocytopenia, yet severe supply shortages persist due to the short shelf life and storage constraints, exacerbating global challenges. Understanding megakaryocyte (MK) development and the triggers that facilitate platelet release will greatly advance human medicine. The exact mechanism and mediators that drive thrombopoiesis remain elusive. Here, we are investigating the role of the extracellular matrix (ECM) in platelet production, with a focus on glycosaminoglycans (GAGs). We developed non-saccharide GAG mimetics (NSGMs) to mimic the activity of natural heparan sulfate. The distinct NSGM G4.2 specifically binds the megakaryocyte receptor G6b-B, enhancing megakaryocyte maturation and differentiation by 200% and increasing platelet production by ~20-fold. However, in megakaryocytes lacking G6b-B expression, there was no effect, confirming that G6b-B is the functional target. In vivo, G4.2 promotes myeloid lineage development by selectively expanding megakaryocyte-primed long-term hematopoietic stem cells and progenitors, including myeloid-biased multipotent progenitors 2, megakaryocytic-erythroid

progenitors, and immature megakaryocyte precursors (MkPs). In an acute thrombocytopenia model, G4.2 significantly accelerates recovery of MkPs, megakaryocytes, and platelets, potentially offering a novel and straightforward approach to guide hematopoietic fate and enhance thrombopoiesis.

(P-132) Fucosylated Chondroitin Sulfate Inhibits Heparan-6-O Endosulfatase Function and Melanoma Invasiveness

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Melanoma accounts for 1-4% of all skin cancers but is responsible for approximately 90% of skin cancer-related deaths. This lethal shift in melanoma disease progression is driven in part by remodeling of the tumor microenvironment (TME), where altered extracellular matrix composition, growth factor signaling, and cell–matrix interactions promote tumor invasion and metastasis. Heparan sulfate (HS) is a sulfated polysaccharide within the TME that regulates many key biological processes through binding and sequestering growth factors and other extracellular ligands. HS is post-synthetically remodeled at the cell surface by the secreted extracellular endosulfatases, SULF1 and SULF2, which selectively remove 6-O-sulfate groups important for ligand binding. Our previous work demonstrated that reducing SULF1 expression reduces melanoma invasion and metastasis in vivo, thus identifying SULF1 as a potential therapeutic target. Despite the key pathophysiological roles of SULF1, no selective small molecule inhibitors exist. Here, we investigated a fucosylated chondroitin sulfate derived from Holothuria floridana (HfFucCS) as a potential inhibitor of SULF1 activity in melanoma. Previous work has shown HfFucCS-mediated inhibition of SULF1 in vitro; thus, we hypothesized that HfFucCS could pharmacologically modulate HS sulfation at the cell surface and attenuate melanoma progression. Human melanoma cells treated with HfFucCS displayed enhanced cell surface HS 6-O-sulfation and increased 6-O-sulfation-dependent FGF1 binding. HfFucCS treatment further reduced migration and invasion in a SULF1-dependent manner. We are currently defining the downstream signaling pathways affected by HfFucCS and determining whether these effects extend across BRAF- and NRAS-mutant melanoma models. Together, these findings support pharmacological modulation of HS remodeling enzymes in the TME as a potential strategy to limit melanoma progression while circumventing the challenges of intracellular drug delivery.

TOPIC 16: Plant Glycobiology, Polysaccharides, and Applications

(KEY16-001) Informing plant cell wall glycan structural models through studies of pectin synthesis - Arabidopsis GALACTURONOSYLTRANSFERASE (GAUT) 1 as a prototype for the “Multiple Pectin Heteroglycan Model” of cell wall architecture

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Plant growth and development rely on cell expansion and wall extensibility that are linked to the pectic polysaccharides [1]. Pectins are homoglycans of homogalacturonan (HG) and multi-domain heteroglycans of HG linked to one or more domains of rhamnogalacturonan II (RG-II) or rhamnogalacturonan I (RG-I) [2]. The number, type, organization, and function of the different pectic heteroglycans in plant walls is not well understood. HG is synthesized by members of the galacturonosyltransferase (GalAT) (GAUT) family. Arabidopsis GAUT1 is an HG biosynthetic alpha-1,4-GalAT [3] that functions in a protein complex with GAUT7 in vivo [4]. The GAUT1:GAUT7 complex both elongates HG acceptors into high molecular weight glycans and initiates HG synthesis from UDP-GalA in the absence of exogenous acceptors [5]. Five other members of the 15-member Arabidopsis GAUT

gene family are biochemically confirmed HG GalATs [6,7], leading to the question of why plants have a large gene family to synthesize this seemingly simple homoglycan. Arabidopsis homozygous gaut1 T-DNA insertion mutants are severely stunted seedlings due to significantly smaller cell size. No mature homozygous plants are recoverable. The mutation impedes pollen tube elongation and hinders normal seed development in heterozygous plants. Complementation of homozygous gaut1 with GAUT1 coding sequence driven by the GAUT1 promoter restores WT-like growth. Homozygous gaut1-1 suspension cells are smaller than WT with ~30% reduced GalAT activity. Analysis of wall extracts revealed reduced GalA content specifically in the 4M KOH post-chlorite fraction, indicating that GAUT1-synthesized HG is held tightly in the wall by hydrogen bonding or oxidation-sensitive linkages. Gel electrophoresis of endopolygalacturonase hydrolyzed extracts identified an HG-associated doublet band (identified as RG-II via NMR analysis) as markedly downregulated in the homozygous gaut1-1 4M KOH post-chlorite fraction. Super resolution microscopy using anti-HG antibodies revealed reduced number and length of HG nanofilaments in homozygous gaut1-1 hypocotyl epidermal and callus cells compared to WT, linking GAUT1-synthesized HG to cell expansion. The results show that GAUT1 synthesizes an HG domain connected to RG-II in a pectic heteropolymer tightly integrated into the wall and required for formation of pectin nanofilaments and cell expansion. The findings support a unique structural and functional role for GAUT1-synthesized HG in planta providing evidence for the Multiple Pectin Heteroglycan Model of cell wall structure and function. (1) Delmer et al. (2024) Plant Cell 36: 1257–1311 (2) Mohnen et al. (2024) In: The plant cell wall - research milestones and conceptual insights. p. 94–126. (3) Sterling et al. (2006) PNAS 103, 5236-41 (4) Atmodjo et al. (2011) PNAS 108, 20225-30 (5) Amos et al. (2018) J. Biol. Chem. 293,19047-63 (6) Biswal et al. (2018) Nat. Biotechnol. 36, 249-257 (7) Engle et al. (2022) Plant J. 109:1441-56

(KEY16-002) Initiation and Elongation of Pectic Homogalacturonan Synthesis on Rhamnogalacturonan by Glycosyltransferases GAUT13 and GAUT14

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Plant cells are surrounded by a dynamic, structurally strong yet malleable extracellular matrix of interacting polymers, including cellulose, hemicellulose, pectin, diverse glycoproteins, and in some cell types, the polyphenolic lignin. Pectin is the most structurally and functionally complex of the cell wall glycans with roles in cell expansion, cell wall structure, cell-to-cell adhesion, pollen tube growth, and plant development. Pectin is a family of galacturonic-acid-rich plant cell wall complex polysaccharides and proteoglycans comprised of the glycan domains homogalacturonan (HG), rhamnogalacturonan I (RG-I), and the substituted HGs rhamnogalacturonan II (RG-II), xylogalacturonan and apiogalacturonan. The number, type, and arrangement of the pectic domains in different pectic polymers is unknown, as is the identity of the enzymes that link the domains together. We show that two Arabidopsis α-1,4-galacturonosyltransferases (GAUTs), GAUT13 and GAUT14 (GT8 family), in addition to previously reported HG acceptor-dependent and HG de novo synthesis activities, initiate and elongate HG on the non-reducing end of RG-I, identifying the first enzymes that synthesize pectic RG-I:HG heteroglycans. While other catalytic GAUTs do not initiate HG production on RG-I, they do elongate HG once initiated on RG-I by GAUT13 and GAUT14. The three different GAUT14 acceptors (UDP-GalA, HG, and RG-I) were shown to compete for the same GAUT14 binding pocket, and amino acids required for GAUT14 catalytic activity were identified using in vitro enzyme assays, computational modeling, and site-directed mutagenesis. We show that a motif RTFASKLQSRS withing GAUT14 hypervariable 1 (HV1) region is necessary and sufficient to supply GAUT10 with RG-I:HG extension activity, providing a novel example of functional glycosyltransferase domain swapping. Recovery of all three catalytic activities in a GAUT13/14 homolog from the non-vascular seedless plant Physcomitrium establishes an early evolution of GAUT13/14 trifunctionality. Together, these findings establish GAUT13 and GAUT14 as RG-I:HG heteroglycan-biosynthetic glycosyltransferases and demonstrate that a small sequence motif in a GT hypervariable domain can reprogram acceptor specificity and confer gain-of-function in GT-A fold enzymes.

(PT-026) Exploring the Biosynthesis of Pectic Rhamnogalacturonan I (RG-I) and its Biological Function in Arabidopsis Embryo Development

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Rhamnogalacturonan I (RG-I) is a pectic polymer with biological roles in cell:cell adhesion, cell wall structure, and plant growth. The RG-I backbone is a disaccharide repeat of galacturonic acid (GalA) and rhamnose (Rha) [-4-α-D-GalpA-1,2-α-L-Rhap-1-] whose synthesis is catalyzed by RG-I:GalATransferase (RGGAT) and RG-I:RhaTransferase (RRT). The first identified RG-I:GalAT, RGGAT1 (At1g28240), is the founding member of the Arabidopsis GT116 family. Based on sequence similarity, we hypothesized that the seven other RGGAT1 homologs in the GT116 family might also exhibit RG-I:GalAT activity with tissue- and/or acceptor-specific functions. To test this, we heterologously expressed RGGAT1 homologs in HEK293 cells and assessed their RG-I:GalAT activity. We identified four additional GT116 family members (RGGAT 2, 3, 4, 7) as RG-I:GalATs. Tests of acceptor specificity showed all five confirmed RGGATs to prefer RG-I acceptors with rhamnose at the non-reducing end and with degrees of polymerization of 10 or greater. We also confirmed three additional RRTs from the Arabidopsis GT106 family as RG-I rhamnosyltransferases. The ability of the five RGGATs to in vitro polymerize the RG-I backbone in combination with an RRT was demonstrated. To probe the biological function of RG-I, we focused on the embryo mutant emb2756, a T-DNA insertion mutant in At1g34550 (RGGAT4). In a large-scale screen of an Arabidopsis heterozygote T-DNA insertion mutant population, emb2756 was identified as a potential embryo-defective mutant with embryo arrest at the globular and heart-shaped stages. These results suggest a possible role for RG-I in embryogenesis. To study this further, we analyzed an emb2756 segregating population of 275 plants, which yielded only four homozygous mutants. These results confirmed that the emb2756 mutation negatively impacted plant growth between seed development and seed germination stages. Furthermore, when seed germination was tested, only 40% of the homozygous seeds germinated, confirming an embryo development or seed germination defect. Homozygous emb2756 mutants also exhibited reduced stem height and rosette diameter, and cell wall analyses revealed an increased GalA content and decreased Rha content, suggesting disrupted RG-I biosynthesis. To determine whether the insertion of the T-DNA in intron 7 of RGGAT4 affected RGGAT4 transcript expression, three-month-old root, rosette leaf and stem tissues of homozygous plants were analyzed. Quantitative PCR analysis revealed a ~90% reduction in RGGAT4 transcript. CRISPR/Cas9-mediated mutagenesis and molecular complementation to validate the emb2756/rggat4 mutant phenotypes are underway. If confirmed, these findings will corroborate a function of RGGAT4-synthesized RG-I in embryogenesis and provide toolbox of RG-I biosynthetic enzymes for probing the different biological functions of RG-I.

(P-133) Evaluating Glycan Remodeling in the Peri-Arbuscular Membrane (PAM) formed in Sorghum Root Cortex during Arbuscular Mycorrhizal Fungi (AMF) Symbiosis

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Arbuscular mycorrhizal fungi (AMF) are endosymbiotic fungi that associate with over 90% of vascular plant roots. Sorghum bicolor is the world's 5th most important cereal crop and has been cultivated globally for thousands of years. AMF facilitate bidirectional exchange of essential micronutrients for host photosynthates across a shared cell wall interface called the peri-arbuscular membrane (PAM). Previously, the range of cell wall polysaccharides identified at the PAM interface have been low due to the limited number of immunogold-labeled glycan-directed antibodies used for their detection. Our goal is to understand how AMF intraradical hyphae penetrate the sorghum root cortex and become enveloped by the PAM without compromising host cell wall integrity. We hypothesize that apoplastic PAM materials in sorghum roots originate from a host driven remodeling response to AMF colonization. We propose that root cortex cells utilize plant cell wall degrading and biosynthetic enzymes to loosen pre-existing cell wall polysaccharides and build new ones in the PAM, resulting in controlled remodeling of the wall that permits simultaneous AMF hyphal penetration and evasion of the plant host cell damage response. To test this hypothesis in a controlled microenvironment, we are developing a robust AMF – sorghum symbiosis monoaxenic in vitro system using sorghum hairy root organ cultures (ROC). To establish hairy root cultures, we have developed a GFP-containing Rhizobium rhizogenes strain ATCC15834, responsible for transmitting hairy root-inducing genetic material, under the control of a constitutive cauliflower mosaic virus (CMV) promoter. Immature sorghum embryos from inbred line Btx623 were co-cultivated with R. rhizogenes strain ATCC15834 forming transgenic calli intended for somatic embryogenesis and eventual hairy root regeneration. The calli produced were excited via epifluorescence microscopy to detect transgenic CMV-GFP expression. Separately, in an effort to progress downstream applications of the ROC, cross sections of in vivo cultivated sorghum roots were generated, fixed and embedded for immunofluorescence staining. CCRC glycan directed antibodies

reactive against a collection of sorghum host cell wall polysaccharides (from CarboSource) were used to profile cell wall carbohydrates within the PAM. Wheat germ agglutinin (WGA), which targets chitin in AMF cell walls, was used to locate arbuscules within the cortical layers. We confirmed that an arabinogalactan II directed monoclonal antibody was reactive against sorghum host cell walls. We also confirmed that the WGA reacts against chitin in AMF intraradical hyphae. Once both systems are established, glycan immunolabeling of synchronized sorghum ROCs will be used to study cell wall remodeling at the PAM fungal-sorghum interface. This study will provide insight into the role of sorghum cell wall remodeling during AMF colonization.

(P-134) Elucidating The Structural Complexity of Rice Cell Wall Polysaccharides: Impact on Saccharification Efficiency and Plant Immunity

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Rice (Oryza sativa), a staple crop feeding billions of people and offers lignocellulosic residue which are rich in carbon polysaccharides for renewable bioenergy and biomaterials conversion. Cellulose, glucuronoarabinoxylan, mixed linkage glucan, lignin, and silica are major cell wall components imparting strength and recalcitrance to enzymatic saccharification in rice. Overall goal of this study is to understand the cross-link framework of the rice cell wall components and their dual roles in saccharification efficiency and plant immunity by studying genotypes (12) with distinct phenotype and in different tissue types. For lignocellulosic biomass evaluation, Asian rice genotypes were used, exhibiting diverse phenotypic characteristics in crop duration and yield. Fourier Transform Infrared(FT-IR) screening resulted in structural differences across tissues; highlighting internodes that showed greatest diversity and utilized for further analyses. Cellulose content was higher in wild genotypes, while high-yielding major genotypes exhibited lower cellulose content, quantified using the Updegraff method. Carbon flux changed because rice genotypes with higher cellulose content showed lower non-cellulosic glucan content, and vice versa. Higher lignin and xylan content was quantified in the high-yielding rice genotype, while mega rice varieties exhibit lower lignin and xylan content. Short-duration rice genotypes had higher xylan content. Higher arabinose and acetyl substitution were observed in 4-month-old rice genotypes as compared to 3-month-old rice plants in short-duration rice genotypes. Moreover, arabinose substitution was higher than acetyl substitution. Out of twelve different rice genotypes, we found long-duration genotypes displayed higher saccharification yield in both conditions, with and without pre-treatments of AIR1. This is the first study reporting the negative correlation between lignin, xylan, acetyl, and arabinose content along with glucose release or cellulose digestibility, thus suggesting that these are the major recalcitrance factors for rice residue degradation and displayed their effect in plant immune responses. These traits may be targeted in the future using genome editing techniques. Additionally, as Xylan-derived Oligosaccharides (XOS) can act as elicitors, we observed whether XOS generated from specific rice varieties with different side-group compositions can show distinct elicitor properties. For evaluation, we enzymatically digested xylans from selected rice genotypes, and the yielding oligosaccharides were applied to rice or Arabidopsis seedlings, which were used to evaluate the tested effect on plant immunity. Our initial investigations suggest that XOS treatment enhances expression of immunity-related genes and increases root growth. Our study identifies elements to enhance saccharification efficiency and reinforces xylan as a biostimulant for plant immunity.

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