LATE-BREAKING POSTER LISTING

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All Members of the Glycosyltransferase-C Superfamily Have a Conserved Membrane Topology
Hans Bakker, Andreia Albuquerque-Wendt, Hermann J. Hütte, Falk FR Buettner, Françoise H. Routier;
Institute of Clinical Biochemistry, Hannover Medical School, Germany;

Poster #: B164 (presented @ PS1) || Abstract #: 187
Changes in gut mucin glycosylation induced by mucin-degrading bacteria promotes colon tumorigenesis
Lubor Borsig, Jesus F. Glaus Garzon;
University of Zurich, Switzerland;

Poster #: B165 (presented @ PS2) || Abstract #: 188
Gelling mechanism of RG-I enriched citrus pectin: Role of arabinose side-chains in cation- and acid-induced gelation
Shiguo Chen[1], Jiaqi Zheng[1], Jianle Chen[1], Hua Zhang[1], Dongmei Wu[1], Xingqian Ye[1], Robert J. Linardt[2];
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Poster #: B166 (presented @ PS1) || Abstract #: 189
Cell Wall Glycosyl Hydrolytic Enzymes Increase Antimicrobial Drug Activity Against Mycobacterium
Lingyi Lynn Deng, Matthew Bo Au; Cristofer Barry, Joshua N. Gustine
Department of Medicine, Boston University School of Medicine, Boston, Massachusetts 02118, USA;

Poster #: B167 (presented @ PS2) || Abstract #: 190
Role of Sialyation in GBM
Sajina GC, Chatherine Libby, Asmi Chakraborty, Brent Jones, Susan Bellis, Anita Hjelmeland;
University of Alabama at Birmingham;

Poster #: B168 (presented @ PS1) || Abstract #: 191
Glycan-Checkpoint Inhibitor unleashing CD8+ T cells against Cancer
Quentin Haas[1], Kayluz F. Boligan[1], Camilla Jandus[2], Cedric Simillion[3], Christoph Schneider[1], Michal Stanczak[4,5], Monika Haubitz[6], Morteza Jafari[7], Alfred Zippelius[4,5], Heinz Läubli[4,5], Robert E. Hunger[7], Pedro Romero[2], Hans-Uwe Simon[1], Stephan von Gunten[1];
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Poster #: B169 (presented @ PS2) || Abstract #: 192
Interaction of viral glycans with heterocomplex of C-type lectins are critical in the pathogenesis of viral infections
Shie-Liang Hsieh,
Genomics Research Center/Academia Sinica;

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CAR-T Cells Targeting a Cancer-Specific, Glycosylated Epitope of Fibronectin Exhibit Potent Anti-Tumor Activity
Tiffany R. King, Fang Liu, Brittany L. Gardner, Avery D. Posey, Jr.;
Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania;

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Suppression of terminal N-glycan modifications by bisecting GlcNAc
Yasuhiko Kizuka,
Center for Highly Advanced Integration of Nano and Life Sciences (G-CHAIN), Gifu University;;

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Generation of a Human Organotypic Skin Model to Study Functions of Glycosaminoglycans
Asha M. Rudjord-Levann[1], Sally Dabelsteen[2], Richard Karlsson[1], Yen-Hsi Chen[1], Rebecca Miller[1], Hans H. Wandall[1];
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Poster #: B173 (presented @ PS2) || Abstract #: 196
Rapid mapping of glycoprotein structure-activity relationships by shotgun scanning glycomutagenesis
Mingji Li, Xiaolu Zheng, Matthew DeLisa;
Robert F. Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY 14853 USA;

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A Recent Advancement Making O-Glycan Preparation Flawless
Yoshiaki Miura[1], Midori Sakaguchi[1], Masaaki Toyoda[1], Akihiko Kameyama[2];
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A carbohydrate mimetic peptide with binding specificity to the Annexin A1 N-terminus overcomes the blood-brain-barrier
Motohiro Nonaka[1], Michiko N. Fukuda[2];
[1]Human Health Sciences, Graduate School of Medicine, Kyoto University, Japan; [2]Cancer Center, Sanford-Burnham-Prebys Medical Discovery Institute, La Jolla, CA;

Poster #: B176 (presented @ PS1) || Abstract #: 199
A bi-to-mono CRD transition in GAL-9 potentiates mesenchymal invasion of breast cancer epithelia
Dharma Pally[1], Anagha Srinivas[1], Rekha V. Kumar[2], Ramray Bhat[1].
[1]Indian Institute of Science, Bangalore, India. [2]Kidwai Cancer institute, Bangalore, India.

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O-Linked Glycopeptides as CNS Penetrant Drugs for the Treatment of Neurodegenerative Diseases and Stroke
Robin Polt[1,2], Michael L. Heien[1,2], John Streicher[1,2];
[1]Teleport Pharmaceuticals, LLC; [2]The University of Arizona;

Poster #: B178 (presented @ PS1) || Abstract #: 201
NMR and MD Evidence for a Mechanistically Important Conformation Change in ST6Gal1
James H. Prestegard, Kelley W. Moremen, Gordon R. Chalmers, Alexander Eletsky, Laura C. Morris, Monique J. Rogals, Robert V. Williams, Jeong-Yeh Yang;
University of Georgia, Athens, GA USA;

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β4galt1 regulates expression of proto-oncogenes PIM-1/2 and Myc in hematopoietic stem cells.
Leonardo Rivadeneyra, Melissa Lee-Sundlov, Robert Burns, Simon Glabere, Heather Ashwood, Karin M. Hoffmeister;
Translational Glycomics Center, Blood Research Institute, Versiti Wisconsin, Milwaukee, WI, USA. ;

Poster #: B180 (presented @ PS1) || Abstract #: 203
The structure of GalNAc-T12 reveals the molecular basis of its substrate recognition mode
Amy J. Fernandez[4], Earnest James Paul Daniel[2], Sai Pooja Mahajan[3], Jeffrey J. Gray[3,4], Thomas A. Gerken[2,5], Lawrence A. Tabak[1], Nadine L. Samara[6];
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Poster #: B181 (presented @ PS2) || Abstract #: 204
IgE Glycosylation Modulates Allergic Inflammation
Kai-Ting C. Shade, Robert M. Anthony;
Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy, and Immunology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129;

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Extracellular vesicles from CLEC2-activated platelets enhance dengue virus-induced lethality via CLEC5A/TLR2
Pei Shan Sung;
Genomics Research Center/Academia Sinica;

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Investigating the Role of α2,3 sialylation and poly-LacNac Structures in Cancer Stem Cell Function
Melanie Walker, Lara K. Mahal, Barbara A. Bensing, Arthur M. Mercurio;
University of Massachusetts Medical School;

Poster #: B184 (presented @ PS1) || Abstract #: 207
GRASP55 senses energy and nutrient deprivation through O-GlcNAcylation to promote autophagosome-lysosome fusion
Xiaoyan Zhang, Leibin Wang and Yanzhuang Wang
University of Michigan, Ann Arbor, MI 48109, USA;

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ST6GAL1 -mediated sialylation in intestinal homeostasis and maintenance of microbiome
Tianxin Yu, Joseph Lau;
Roswell Park Cancer Institute, Buffalo, NY, USA;

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MotifFinder, Managing the Glycomics Headache
Jian Zhang[1], Zachary Klamer[2], Jonathan Beirne[1], Xi Chen[1], Brian Haab[2];
[1]Z Biotech, LLC, Aurora, Colorado; [2]Center for Cancer and Cell Biology, Van Andel Research Institute, Grand Rapids MI;

Poster #: B187 (presented @ PS2) || Abstract #: 210
Interactions of Tau and Heparin/GAGs
Fuming Zhang[1], Jing Zhao[2], Chunyu Wang[2], Robert J. Linhardt[3];
[1]Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute; [2]Department of Biology, Rensselaer Polytechnic Institute; [3]Departments of Chemistry and Chemical Biology and Biomedical Engineering, Rensselaer Polytechnic Institute;

Poster #: B188 (presented @ PS1) || Abstract #: 211
Regulation of Extrinsic Glycosylation in Platelets
Jinchao Zhang, Joseph Lau;
Roswell Park Cancer Institute, Buffalo, NY, USA;

LATE-BREAKING ABSTRACTS

(186) All Members of the Glycosyltransferase-C Superfamily Have a Conserved Membrane Topology

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Glycosyltransferases that use polyisoprenol-linked donor substrates are categorized in the GT-C superfamily. In eukaryotes, they act in the endoplasmic reticulum (ER) lumen and are involved in N-glycosylation, glypiation, O-mannosylation, and C-mannosylation of proteins. The membrane topology model of C-mannosyltransferases (DPY19 family) concurres perfectly with the 13 transmembrane domains (TMDs) observed in oligosaccharyltransferase (STT3 family) structures. A multiple alignment of family members from diverse organisms highlighted the presence of only a few conserved amino acids between DPY19 and STT3. Most of these residues were shown to be essential for DPY19 function and are positioned in luminal loops that showed high conservation within the DPY19 family. Multiple alignments of other eukaryotic GT-C families underlined the presence of similar conserved motifs in luminal loops, in all enzymes of the superfamily. Most GT-C enzymes are proposed to have an uneven number of TDMs with 11 (POMT, TMTC, ALG9, ALG12, PIGB, PIGV, and PIGZ) or 13 (DPY19, STT3, and ALG10) membrane spanning helices. In contrast, PIGM, ALG3, ALG6, and ALG8 have 12 or 14 TMDs and display a C-terminal dilysine ER-retrieval motif oriented towards the cytoplasm. We propose that all members of the GT-C superfamily are evolutionary related enzymes with preserved membrane topology with a basis of 11 TMDs to which several members obtained additional ones.

(187) Changes in gut mucin glycosylation induced by mucin-degrading bacteria promotes colon tumorigenesis

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Host cells in the gastrointestinal tract live in a mutualistic relationship with gut microbiota in healthy condition. The mucus layer represents the interface that both creates a physical barrier and forms a niche for microbiota thereby contributing to gut homeostasis. Alteration of gut microbiota has been associated with several diseases, including bowel inflammatory disease and cancer. While changes in cell surface glycosylation are typical hallmarks of tumorigenesis, a direct involvement of bacteria-derived glycolytic enzymes in cancer remains to be defined. We tested the hypothesis that a prominent mucin-degrading bacteria *Akkermansia muciniphila* may affect the progression of colorectal tumors.

First, we tested whether the glycolytic activity of *A. muciniphila* alters glycosylation of a panel of tumor cells. We observed changes in lectin binding to treated tumor cells, which was in agreement with the presence of sialidase, N-acetylglucosaminidase, and galactosidase activity. Colonization of mice with *A. muciniphila* resulted in altered levels of free monosaccharides in the cecum fluid, with a prominent increase in free sialic acid, and changes in microbiota. In addition, significant changes in SCFA in the colon has been observed. Cecum implantation of MC-38 tumor cells in *A. muciniphila* colonized mice resulted in enhanced tumor growth and altered tumor immunity. The ongoing characterization of colonic mucins indicate changes in glycosylation. Taken together, these data indicate that enhanced mouse colonization with mucin-degrading bacteria affects tumor growth.

(188) Gelling mechanism of RG-I enriched citrus pectin: Role of arabinose side-chains in cation- and acid-induced gelation

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RG-I enriched pectin is present in fruit and vegetable containing products. However, it is removed by the hot acid treatment during commercial pectin production to improve gelling properties and to afford a more uniform pectin quality. Recently, an awareness of the health benefits of RG-I enriched pectin has caused technologists to rethink its utilization by the food industry, especially as a novel healthy gelling agent. Unique RG-I enriched pectin with abundant arabinan side-chains was extracted from citrus membrane by sequential mild acidic and alkaline treatment. Arabinose was then removed by enzymatic treatment to investigate the impact of arabinose side-chains on gelation. The properties of RG-I enriched pectin gels, prepared using cations or acid, showed it could form gels under conditions required for both low and high methoxyl pectin as a result of its highly branched structure. In cation-induced gelation, the HG region forms egg-box junction zones with divalent cations and the side-chains of the RG-I region stabilizes the network structure through entanglements. In acid-induced gelation, low pH promotes formation of hydrogen bonding and hydrophobic interactions within the HG region and the side-chains create a tighter conformation, eventually allowing for stronger interactions between the pectin chains.

(189) Cell Wall Glycosyl Hydrolytic Enzymes Increase Antimicrobial Drug Activity Against Mycobacterium

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Cell wall glycosyl hydrolases are enzymes that cleave bacterial cell walls by hydrolyzing specific bonds within peptidoglycan and other portions of the envelope. Many organisms possess glycosyl hydrolases. This study specifically investigated whether cell wall glycosyl hydrolytic enzymes could be employed as exogenous reagents to augment the efficacy of antimicrobial agents against mycobacteria. *Mycobacterium smegmatis* cultures were treated with thirty conventional drugs (mostly antibiotics) and six anti-tuberculosis drugs – alone or in combination with cell wall hydrolases. Culture turbidity, colony-forming units (CFUs), metabolic assays (BioLog), vital staining, and oxygen consumption were all monitored. The majority of antimicrobial agents tested alone only had minimal inhibitory effects on bacterial growth. However, the combination of cell wall hydrolases and most of the antimicrobial agents tested, revealed a synergistic effect that resulted in significant enhancement of bactericidal activity. Vital staining showed increased cellular damage when *M. smegmatis* and *Mycobacterium bovis* bacillus Calmette–Guérin (*M. bovis* BCG) were treated with both drug and lysozyme. Respiration analysis revealed stress responses when cells were treated with lysozyme and drugs individually, and an acute increase in oxygen consumption when treated with both drug and lysozyme. Similar trends were also observed for three other enzymes

(hydrolase-30, RipA-His_6 and RpfE-His_6). These findings demonstrated that cell wall glycosyl hydrolytic enzymes have the capability to improve the potency of many current antimicrobial drugs and render some ineffective antibiotics effective in killing mycobacteria. This combinatorial approach may represent an important strategy to eliminate drug-resistant bacteria.

(190) Role of Sialyation in GBM

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Glioblastoma (GBM) is one of the most aggressive and fatal cancers with a median survival of only 14 months with current standard of care which includes maximal surgical resection, radiation and chemotherapy. Despite available treatments, GBM is incurable with rapid recurrence and low life expectancy. Moreover, development of effective treatments is difficult due to the highly heterogeneous nature of GBM caused by brain tumor initiating cells (TICs), which exhibit stem cell-like capacity of self-renewal, multilineage differentiation, and tumorigenicity. Brain TICs are resistant to radio- and chemotherapy and thought to cause tumor recurrence. Therefore, it is highly imperative to understand the mechanisms promoting BTIC maintenance to develop new treatments for GBM. How BTIC maintenance is regulated by post-translational modifications like glycosylation is understudied. Altered cell surface glycosylation was one of the earliest modifications observed in malignant neoplastic progression. However, this facet of brain tumor biology has not received particular attention. Among the various glycosyltransferases present in human cells, *golgi* sialyltransferase ST6Gal-I (beta-galactoside alpha-2,6-sialyltransferase 1) adds sialic acid residues in α2-6 linkage to membrane bound and secreted N-glycans. Through this modification, ST6Gal-I is an important driver of tumorigenic processes such as epithelial to mesenchymal transformation, TIC maintenance, tumor cell resistance to apoptotic stimuli, radio- and chemoresistance and increased survival of cells exposed to stressors such as hypoxia and serum starvation in various cancers such as pancreatic and ovarian cancer. While roles of ST6gal-I have not been explored in brain TICs, we hypothesize that *ST6Gal-I mediated silalylation of surface receptors in GBM promotes stemness.* Our data with GBM patient derived xenografts (PDX) depicts increased stemness with high α2,6 sialylation, while, ST6Gal-I knockdown (KD) in GBM PDX lines show decreased cell growth. These findings strongly implicate ST6Gal-I mediated in BTIC maintenance and GBM tumorigenesis. Determining the mechanistic basis of sialylation-dependent maintenance of BTICs will highlight a novel insight in GBM stemness.

(191) Glycan-Checkpoint Inhibitor unleashing CD8+ T cells against Cancer

Quentin Haas[1], Kayluz F. Boligan[1], Camilla Jandus[2], Cedric Simillion[3], Christoph Schneider[1], Michal Stanczak[4,5], Monika Haubitz[6], Morteza Jafari[7], Alfred Zippelius[4,5], Heinz Läubli[4,5], Robert E. Hunger[7], Pedro Romero[2], Hans-Uwe Simon[1], Stephan von Gunten[1]

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Cytotoxic T lymphocytes (CTL) play a key role against cancer. Siglecs are inhibitory receptors recognizing sialoglycans and are able to trigger inhibitory functions on immune cells. The ligands of Siglec receptors are highly expressed in various types of tumors, developing a coat of sialic acid on their surface.

We hypothesized that Siglec expression on $CD8^+$ T cells is used by sialic acid-coated tumor cells to bypass immune cell recognition. Thus, we investigated $Siglec^+$ $CD8^+$ T cells characteristics in healthy donors as well as in tumor infiltrating lymphocytes (TILs) from patients with melanoma. $CD8^+$ T cells functional capacities were analyzed, as well as the pattern of clonality and expansion of $Siglec^+$ $CD8^+$ T cells in melanoma. Beside, experiments performed on patients' material were corroborated with biostatistical analyses of cancers RNA databases. In complement, Siglecs ligands expression in melanoma was also quantified.

Our results show an extended $Siglec^+$ $CD8^+$ T cell pool in the tumor infiltrating lymphocytes (TILs) isolated from patients with melanoma compared to healthy donors. The $Siglec^+$ $CD8^+$ T cell pool represent a more differentiated, more cytotoxic and more proliferative subset of $CD8^+$ T cell. We also demonstrated that these effector capacities were dampened upon ligation of Siglec with its ligands. This inhibitory capacity of Siglecs could be canceled when the ligands were removed from cancer cells surface.

Our data suggest that Siglecs on $CD8^+$ T cells may represent a novel potential therapeutic targets for immune check-point therapy of malignancies with high expression of sialoglycans, such as melanoma.

(192) Interaction of viral glycans with heterocomplex of C-type lectins are critical in the pathogenesis of viral infections

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Previous studies of host-pathogen interactions are from the view point of single PAMP (pathogen-associated molecular pattern)-PRR (pattern recognition receptor) contact. However, pathogens carry multiple PAMPs and are able to activate multiple innate immunity receptors simultaneously upon engagement with immune cells. It has been demonstrated that dengue virus (DV) and influenza virus (H5N1) interact with CLEC5A, DC-SIGN, DC-SIGNR, and mannose receptor. Compared to DC-SIGN and DC-SIGNR, the affinity between viruses and CLEC5A is much lower. Nevertheless, only CLEC5A has clear biochemical evidence to trigger downstream signaling pathway after engagement, while the cytoplasmic domains of DC-SIGN, DC-SIGNR, and MR do not have well-defined domains for signal transduction. We demonstrate that viral glycans interact with heterocomplex of C-type lectins in macrophages, neutrophils, and platelets, thus enhances virus-induced inflammatory cytokine production and extracellular vesicles release via Syk-coupled C type lectins – CLEC5A and CLEC2. All these observations suggest that blockade of Syk-coupled C type lectins is a promising approach to attenuate inflammatory reactions and reduce lethality in acute viral infections.

(193) CAR-T Cells Targeting a Cancer-Specific, Glycosylated Epitope of Fibronectin Exhibit Potent Anti-Tumor Activity

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Current immunotherapy advances have been revolutionary for the treatment of hematologic malignancies as evident by the FDA approvals of CD19-targeting CAR-T cells for the treatment of acute lymphoblastic leukemia and diffuse-large B-cell lymphoma. However, the greatest unmet burden for cancer treatment is solid tumors, particularly prostate, breast, colorectal, and lung cancers, which account for approximately 45% of all cancer related deaths in the U.S. CAR-T cells have lacked efficacy in the fight against solid tumors due to a number of challenges, including the lack of tumor-specific antigens, overcoming obstacles of therapeutic resistance, tumor heterogeneity, poor expansion and persistence, and extrinsic dysfunction and physical barriers to T cell infiltration caused by the dense, immunosuppressive tumor microenvironment (TME). In order to enhance the efficacy of CAR-T cells against solid tumors, post-translational modifications that occur exclusively in transformed cells can be targeted. Aberrant glycosylation is considered a new hallmark of cancer development as glycans play a key role in tumor initiation, progression, and metastasis. Alterations in glycosyltransferases and chaperone proteins lead to the development of various tumor-associated antigens. For example, defects in mucin-type O-glycosylation leads to cell surface expression of terminal GalNAc, or Tn-antigen, on many tumors. Previous pre-clinical studies that target Tn-MUC1 with 5E5-CAR-T cells demonstrated efficacy against multiple tumor histotypes, and these studies have recently translated into a phase I clinical trial (NCT04025216) for the treatment of NSCLC, ovarian cancer, triple-negative breast cancer, pancreatic adenocarcinoma, and multiple myeloma. The present work targets Tn-antigen present on the IIICS domain of oncofetal fibronectin (onfFN), a cancer-specific splice isoform of the extracellular matrix protein (ECM) fibronectin (FN). Current data shows that onfFN-targeting CAR-T cells secrete high concentrations of IFN-γ in response to co-culture with metastatic prostate cancer cells. *In vitro* studies reveal onfFN-targeting CAR-T cells as a potent cytotoxic agent against the androgen-insensitive PC3 and DU145 prostate cancer cell lines at multiple effector-to-target ratios. Additionally, IIICS-FN targeting CAR-T cells promote rapid anti-tumor rejection in a subcutaneous PC3 xenograft model of prostate cancer. Here, we present a strategy to target a cancer-specific glycosylated epitope on an ECM protein found within the TME with CAR-T cells, which demonstrates *in vitro* and *in vivo* efficacy against metastatic prostate tumors. This data provides a novel cancer immunotherapy approach for the treatment of prostate tumors and potentially other cancer histotypes.

(194) Suppression of terminal N-glycan modifications by bisecting GlcNAc

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Biosynthesis of N-glycans is a highly regulated process with stepwise actions of various glycosyltransferases in the Golgi, and it is unclear how each step is inter-regulated and integrated. Bisecting GlcNAc, a central GlcNAc branch synthesized by GnT-III (MGAT3 gene), is highly expressed in brain. Our previous studies using Alzheimer's disease model mice showed that bisecting GlcNAc promotes Alzheimer's pathology by regulating the intracellular location of amyloid beta-producing enzyme BACE1. However, physiological functions of bisecting GlcNAc remain largely unclear. Here we found that bisecting GlcNAc is a general suppressor of various terminal modifications of N-glycans (Nakano et al., *Mol. Cell. Proteomics*, in press).

Previous in vitro enzymatic studies showed that the presence of bisecting GlcNAc inhibits the actions of other N-glycan branching enzymes, such as GnT-IV (MGAT4) and -V (MGAT5), suggesting that bisecting GlcNAc has a big influence on overall N-glycan profiles in vivo. To explore this possibility, we performed N-glycomic analysis of Mgat3-deficient brain using LC-MS and revealed that various types of terminal modifications of N-glycans were aberrantly upregulated in Mgat3-knockout, including Lewis-type fucose, sialic acid and HNK-1 epitopes. The similar results were also obtained in mouse kidney. The mRNA levels of the responsible glycosyltransferases were unaltered in Mgat3-knockout. In contrast, enzyme assays using bisected and non-bisected acceptor oligosaccharides clearly showed that most enzymes acting on N-glycan terminals prefer the non-bisected glycan as a substrate. This indicates that the upregulation of terminal N-glycan epitopes in Mgat3-KO were attributed to the fine substrate specificities of glycosyltransferases. We performed molecular dynamics simulation of glycosyltransferase-acceptor glycan complexes and showed that the presence of bisecting GlcNAc changed N-glycan conformation from an extended type to a back-fold type in which alpha1,6-mannose arm loses interaction with the enzymes. This conformation change is suggested to be the cause for the lower activity of various glycosyltransferases toward the bisected aceptor. In sum, these findings highlight the roles of bisecting GlcNAc as a general suppressor for terminal modifications of N-glycans and provide us with new insights into how protein N-glycosylation is regulated in cells.

(195) Generation of a Human Organotypic Skin Model to Study Functions of Glycosaminoglycans

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Glycosaminoglycans (GAGs) are long, linear, polysaccharide chains of alternating disaccharide units covalently linked to a protein core to form diverse proteoglycans that regulate processes involved in development, growth, aging, tissue regeneration, and cancer. GAGs control these processes through structural motifs specified by chain structure and modifications, which serve as binding sites for growth factors and their cognate receptors. Variations in GAG chain length, disaccharide composition and residue modifications confer a high degree of heterogeneity, which has made it difficult to define the molecular functions of the different GAG motifs in normal tissue formation and regeneration. Here, we employed CRISPR-Cas9 genetic engineering to deconstruct and dissect the molecular functions of the main GAG types in a human tissue model. We have generated a first-generation, 3D, organotypic platform to initiate the systematic dissection of GAG functions in human tissue formation and homeostasis. Our tissue library demonstrates distinct phenotypes with impact on general tissue homeostasis and barrier formation associated with loss of GAG chain initiation and elongation. Our platform provides a contextualized approach to define the functions of specific GAG structures in human epithelial biology with a broad discovery potential.

(196) Rapid mapping of glycoprotein structure-activity relationships by shotgun scanning glycomutagenesis

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N-linked glycosylation serves to diversity the proteome and is crucial for the folding and activity of numerous cellular proteins. Consequently, there is great interest in uncovering the rules that govern how glycosylation modulates protein properties so that the effects of site-specific glycosylation might eventually be predicted. Towards this goal, we describe a combinatorial strategy termed shotgun scanning glycomutagenesis (SSGM) that enables systematic investigation of the structural and functional consequences of glycan installation along a protein backbone. The utility of this approach was first demonstrated with two different model proteins, bacterial immunity protein Im7 and bovine pancreatic ribonuclease A, both of which were found to tolerate *N*-glycan attachment at an unexpectedly large number of positions and with relatively high efficiency. The stability and activity of many glycovariants was measurably altered by the *N*-linked glycan in a manner that critically depended on the precise location of the modification. Next, SSGM was leveraged to identify glycoengineered variants of an anti-HER2 human single-chain Fv (scFv) antibody with enhanced antigen-binding activity. By enabling high-resolution mapping of glycan-mediated effects on acceptor proteins, glycomutagenesis opens up possibilities for accessing unexplored regions of glycoprotein structural space and engineering protein variants with advantageous biophysical and biological properties.

(197) A Recent Advancement Making O-Glycan Preparation Flawless

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It has been widely recognized that glycosylations of proteins are responsible for their functions in many aspects. Therefore, the glycosylation of glycoconjugates are of interest for understanding their structure-function relationships. Analysis of N-linked glycans has been facilitated by means of N glycosidases such as PNGase F, while that of O-linked glycans is left behind due to lack of practical releasing method. Each technique to prepare free O-glycans from glycoproteins appears to possess pros and cons in terms of safety, yield, processing time, higher rate of undesired side reaction (peeling), and so on.

Here we introduce a newly developed research tool for O-glycan analysis of glycoproteins; EZGlyco® O-Glycan Prep Kit. The kit operation will be accomplished within 5 to 6 hours prior to the analysis, allowing one–day O-glycan analysis. The kit utilizes totally a new combination of chemical reagents and O-glycan enrichment bead, enabling a rapid and accurate recovery of O-linked sugars from glycoproteins. The kit generates a minimum amount of peeling products and efficiently liberates Olinked sugars. Sugars are recovered as reducing form so that the kit granted 2-aminobezamide labeling of the recovered carbohydrates for efficient detection with fluorescent detector equipped with LC system such as HPLC, UHPLC, and LC-MS.

Avoiding complicated manipulations and disadvantages of currently available methods, the well-designed kit would finally convince researchers for the choice of O-glycan preparation Kit. In this study, we will present a detailed investigation of the new kit including its robustness and flawless integration in the O-glycan analysis. We believe that the EZGlyco O-Glycan Prep Kit is far more practical choice than any other conventional methods in all aspect.

(198) A carbohydrate mimetic peptide with binding specificity to the Annexin A1 N-terminus overcomes the blood-brain-barrier

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Annexin A1 (Anxa1) is normally expressed intracellularly in numerous cell types. However, in malignant tumors, Anxa1 is found on the cell surface of endothelial cells (Oh *et. al., Nature*, 429: 629-35, 2004), suggesting that it serves as a cell surface marker of tumor vasculature and could be useful for drug delivery. Previously, we identified a series of Lewis A glycan mimetic peptides using phage display technology. One of them, designated IF7, targeted malignant tumors following intravenous injection, likely via Anxa1 expressed on the tumor endothelial cell surface (Hatakeyama *et.al. PNAS* 108: 19587-92, 2011). Moreover, we observed that intravenously-injected IF7 crossed endothelial cells by transcytosis and penetrated the stroma where tumor cells reside. Based on these observations, we hypothesized that IF7 could overcome blood-brain-barrier (BBB) to function as a drug delivery vehicle for malignant brain tumors. To test this model, we injected fluorescently-labeled IF7 intravenously into glioma tumor model mice and observed accumulation of fluorescence in brain tumor cells, supporting the idea that IF7 crossed the BBB. We then conjugated IF7 with SN38, a biologically active metabolite of irinotecan, and injected the resulting peptide (IF7-SN38) intravenously at low dosage and observed regression of brain tumors in model mice. These results suggest that SN38 conjugated with an Anxa1-binding peptide can overcome BBB and efficiently suppress growth of malignant brain tumors. We have also developed a new series of more stable Anxa1-binding peptides composed of D-amino acids suitable for drug delivery to tumors.

(199) A bi-to-mono CRD transition in GAL-9 potentiates mesenchymal invasion of breast cancer epithelia

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Aberrant expression and functions of glycans and their binding proteins (lectins) represent one of the earliest 'hallmarks' of cancer. Galectins are a conserved family of lectins that can bind to β-galactosides. A special class of galectins known as tandem-repeat (GAL-4, -8, -9, and -12 in humans) can bind two distinct β-galactosides simultaneously and play intricate roles in physiological and pathological contexts. In this study, we asked if one or more tandem repeat galectins regulate breast tumor progression based on earlier reports of their differential expression. Upon mimicking a spectrum of progression from homeostatic breast- to invasive cancerous architectures by culturing HMLE (immortalised breast epithelial cell line), MCF7 (non-invasive breast cancer cell line), MDA-MB-231 (metastatic cell line) in laminin-rich ECM- and Type 1 collagen- rich scaffold gels, we observed that expression of the gene encoding GAL-9 tracked invasiveness of probed cells. Breast cancer patient samples (especially with a 'triple negative' (ER^- /PR^- /$HER2^-$) histotype) showed higher levels of GAL-9 when compared with matched adjacent normal tissues. Perturbing GAL-9 levels in cancer epithelia showed its positive correlation with their adhesion to- and invasion within- laminin-rich matrices. Within a complex bimatrix scaffold that mimics the epithelial-basement membrane-stromal matrix organization, GAL-9 preferentially enhanced the solitary over collective invasion of cancer epithelia. To dissect which carbohydrate recognition domain (CRD) is involved in regulation of cancer invasion, we generated GAL-9 mutants with deletion of individual CRDs or the intervening linker. Only the misexpression of the N-terminal CRD of GAL-9 (and not the C-terminal CRD or linker deletion) is able to increase

cancer invasion similar to full length GAL-9 overexpression. We also observed that GAL-9 in human and murine invasive cancer cells was cleaved into individual CRDs, as opposed to expression of primarily uncleaved biCRD forms in untransformed and non-invasive transformed cells. Our results, in the light of a strongly predicted protease-susceptibility of the GAL-9 linker region suggest that the N-CRD of GAL-9 that is free of the C-CRD potentiates the mesenchymal invasion of cancer epithelia through stromal-like milieu.

(200) O-Linked Glycopeptides as CNS Penetrant Drugs for the Treatment of Neurodegenerative Diseases and Stroke

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Endogenous peptide neurotransmitters related to enkephalins, dynorphins, angiotensins, secretins, and other peptide hormones, both cyclic and linear, have been converted into O-linked glycopeptide drug candidates. Short glycopeptides (5–7 residues) have been created which produce mu opioid agonism, delta opioid agonism, or synergistic mu + delta opioid agonism. By linking helical amphipathic "addresses" to these opioid "messages" it was possible to enhance their anti-nociceptive effects *in vivo* in rodents. Glycosylated angiotensin analogues with neuroprotective activity have been synthesized that show extended stability and blood-brain barrier (BBB) penetration in male rats. Large pituitary adenylate cyclase-activating peptide (PACAP) compounds have also been created with longer linear sequences. These have neuroprotective and neurorestorative potential. Remarkable therapeutic effects are observed in both stroke and TBI models in mice. MSN analysis in conjunction with microdialysis has been used to measure both stability and BBB penetration of these compounds in male rodents. With this advance it is now possible to determine pharmacokinetic profiles for this new class of drugs that are typically cleared from serum by the kidneys. Using this approach, we demonstrate that glycosylated peptide drugs possess enhanced metabolic stability and BBB penetration. Molecular weight (MW) does not appear to affect BBB penetration rates, at least in the range of MW's examined so far, 550—3,500 Daltons. This approach thus has great promise to make even large neuropeptides "drugable." We hypothesize that this ability to penetrate the BBB is due to the ability of the glycopeptides to adopt conformations that render them either highly water soluble or highly amphipathic structures that associate strongly with biological membranes, *e.g.* "biousian behavior."

Random coil conformational ensembles are water soluble for both peptides and glycopeptides. In the presence of membranes, neuropeptides are expected to adopt folded amphipathic conformations that maximize contact of the hydrophobic regions of the peptide to the membrane and hydrophilic regions with water. The native peptides sink deeper into the membrane, and are held more tightly. The introduction of water-soluble carbohydrates at the C-terminus is expected to shift the equilibrium toward the aqueous environment where the glycopeptide can "hop" to another membrane. The result is vastly improved PK/PD properties, allowing the glycopeptides to be used as drugs, whereas the unglycosylated neurotransmitters (hormones) bind to the first membrane they are exposed to, and never reach the site of action in the brain.

(201) NMR and MD Evidence for a Mechanistically Important Conformation Change in ST6Gal1

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It has long been known that the sialyl transferase, ST6Gal1, binds its galactose-terminated acceptors more tightly after its sialyl-CMP donor is bound, but a structural explanation has been lacking. In a continuing effort to provide assigned NMR resonances that can report on various states of this enzyme we ran an extended molecular dynamics (MD) simulation beginning with the crystal structure of the apo form of the rat enzyme, modified to include a segment from a crystal structure of the human, CMP-bound, enzyme that was missing in the apo rat structure. Surprisingly, after ~200ns of simulation there was a dramatic structural change in this loop that closed the commonly accepted entrance to the donor cavity and opened an alternate entrance ~90 degrees from the accepted entrance. An acceptor cannot bind in this alternate conformation, but if binding of a donor were to shift the alternate conformation back to that seen in the CMP-bound conformer, it could provide an explanation for the change in acceptor affinity on donor binding. Evidence supporting this conformation change comes from our recent assignment of crosspeaks in HSQC spectra of a sample expressed in HEK293 cells and isotopically labeled with ^{15}N in all phenylalanine residues. The assignment strategy uses a genetic algorithm search for the best match between observed NMR parameters and parameters predicted from extended MD simulations. Using MD segments from a 1 μs trajectory we find that segments from the end of the trajectory give better assignment scores than segments from the beginning of the trajectory and combining all segments gives the best score. This suggests that the apo form is dynamic but favors a structure with an alternate donor entrance. Additional evidence comes from isotopic labeling the protein with ^{13}C-methyl-methionine. There is a methionine near the missing segment. Interestingly, the ^{13}C-methyl crosspeak for this methionine is spilt in two, supporting the existence of two conformers.

(202) β4galt1 regulates expression of proto-oncogenes PIM-1/2 and Myc in hematopoietic stem cells.

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The quiescence, self-renewal, and fate determination of hematopoietic stem cells (HSCs) is regulated in a concerted fashion by cellular and extrinsic components, including cell adhesion molecules, soluble and membrane-bound factors, extracellular matrix, surrounding cells, and glycans. Type-2 Lactosamines (LacNAc) are structures generated by β1-4 galactosyltransferase type 1 (β4galt1) that regulate homing and migration of HSCs. Here, we further investigated the role of β4galt1 in the regulation of HSCs.

Flow cytometry analysis showed that β4galt1-/- mouse bone marrow samples have increased numbers of Long-Term HSCs (LT-HSC), phenotypically defined as Lineage^{NEG}/cKit^{POS}/Sca-1^{POS}/CD150^{POS}/CD48^{NEG}. Analysis of LT-HSC protein lysate using a 45- lectin microarray showed a decrease in high mannose structures (GNA), α2-3 sialylation (MAL I and ECA) and α1-2 and α1-6 fucosylation (LTL, UEL I and AOL). These changes are consistent with the lack of β4galt1-mediated galactosylation of target acceptors. In contrast, O-glycan structures appeared to be increased as judged by Calsepa and PTL-I lectin binding.

Single cell RNA sequencing (scRNA seq) of sorted Lineage^{NEG}/Sca-1^{POS}/cKit^{POS} cells (LSK) showed a significantly increased expression of the proto-oncogene Pim1/2, its downstream signaling partner nuclear phosphoprotein Myc, and the transmembrane receptor mucin 13 (MUC13) in β4galt1-null cells compared to littermate controls. Pim1 is a serine/threonine kinase whose overexpression has been associated with human tumors, mainly in hematological malignancies. Unbiased gene set enrichment analysis (GSEA) revealed a significant upregulation in the Pim1-associated Jak/Stat pathway. Pro-inflammatory cytokines were not increased in the bone

marrow as determined by cytokine profile analysis. The data suggest that cytokine-induced signaling does not play a role in activating Jak/Stat signaling cascades associated with proto-oncogene Pim1/2 and Myc upregulation in the β4galt1 deficient HSCs. Recent data suggest that MUC13 has oncogenic potential. Thus, the lack of β4galt1-dependent glycosylation is associated with over expression of several proto-oncogenes, including the heavily glycosylated MUC13, in HSCs, thereby affecting HSC function.

(203) The structure of GalNAc-T12 reveals the molecular basis of its substrate recognition mode

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Polypeptide *N*-acetylgalactosaminyl transferases (GalNAc-Ts) initiate mucin type O-glycosylation by catalyzing the transfer of N-acetylgalactosamine (GalNAc) to a Ser or Thr on a substrate. The enzymes do not recognize a consensus sequence or structural motif and the mechanism of substrate binding and recognition is not clear. However, it has been established that the N-terminal catalytic domain and C-terminal lectin domain of GalNAc-Ts can interact with peptide substrates and position them for transfer to a specific Thr or Ser on the substrate. The human isoform GalNAc-T12 is of biomedical interest as inactive and partially active variants are present in subsets of patients with colorectal cancer. Previous biochemical studies of GalNAc-T12 reveal a unique mechanism of substrate recognition that combines both catalytic and lectin domain binding to previously glycosylated sites on a peptide substrate. To understand the molecular basis of substrate recognition, we have solved the X-ray crystal structure of the enzyme bound to a di-glycopeptide substrate at 2.0 Å resolution. The structure reveals a distinct substrate recognition mode that is mediated by non-conserved residues in the catalytic domain of GalNAc-T12.

(204) IgE Glycosylation Modulates Allergic Inflammation

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The prevalence of allergies has markedly increased over the past few decades, constituting a major healthcare problem. Allergies are caused by production of immunoglobulin ε (IgE) antibodies targeting environmental substances, such as pollens and foods. Individuals become sensitized when allergen-specific IgE binds to the high-affinity receptor (FcεRI) on tissue mast cells or blood basophils. Subsequent allergen exposure crosslinks cell-bound IgE, resulting in the release of inflammatory mediators including histamine, leukotrienes, and prostaglandins. Despite its significance in allergic diseases, IgE biology suffers from major knowledge gaps. While IgE is critical for pathogenesis of allergic diseases, some individuals have allergen-specific IgE but do not experience allergic symptoms. Further, some individuals outgrow their allergens, while retaining detecting levels of allergen-specific IgE. As diagnosis of allergy relies on detection of allergen-specific IgE with a clinically-suggestive history, these parameters result in almost 50% false-positive rate for allergies to food. This over-diagnosis of allergies is problematic, leading to treatment consisting of food avoidance which may actually contribute to allergies. Thus, despite its discovery over 50 years ago, it is not clear what make IgE pathogenic and glycosylation is often an overlooked aspect of IgE biology.

The importance of glycosylation for a number of antibody classes has been established. However, the contribution of glycosylation to IgE biology is less clear. IgE are the most heavily glycosylated monomeric antibodies with seven Asparagine (N)-linked glycosylation sites on its constant domains. One site carries exclusively oligomannose glycans (N394), one site is unoccupied (N383), while the remaining sites contain sialylated glycans. Previously we demonstrated that a single N-linked oligomannose structure in the constant domain 3 (Cε3) of IgE, at asparagine-394 (N394) in human IgE is absolutely required in allergic reactions. Genetic disruption of the site or enzymatic removal of the oligomannose glycan altered IgE secondary structure and abrogated IgE binding to FcεRI, rendering IgE incapable of eliciting mast cell degranulation, thereby preventing anaphylaxis. It is appealing to speculate that glycans on IgE explain the presence of allergen-specific IgE and manifestation of allergic symptoms, and may serve as a potential allergic disease biomarker and a novel therapeutic target.

(205) Extracellular vesicles from CLEC2-activated platelets enhance dengue virus-induced lethality via CLEC5A/TLR2

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Platelet-leukocyte interactions amplify inflammatory reactions, but the underlying mechanism is still unclear. CLEC5A and CLEC2 are spleen tyrosine kinase (Syk)-coupled C-type lectin receptors, abundantly expressed by leukocytes and platelets, respectively. CLEC5A is a pattern recognition receptor (PRR) to flaviviruses and binding to terminal fucose and mannose moieties of viral glycans, CLEC5A also binds to N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) disaccharides of bacterial cell walls. CLEC2 is the platelet-specific receptor which binds to its ligand podoplanin via O-glycan-dependent interaction. Here we show that dengue virus (DV) activates platelets via CLEC2 to release extracellular vesicles (EVs), including exosomes (EXOs) and microvesicles (MVs). DV-induced EXOs (DV-EXOs) and MVs (DV-MVs) further activate CLEC5A and TLR2 on neutrophils and macrophages, thereby induce neutrophil extracellular trap (NET) formation and proinflammatory cytokine release. Compared to *stat1*$^{-/-}$ mice, simultaneous blockade of CLEC5A and TLR2 effectively attenuates DV-induced inflammatory response and increases the survival rate from 30 to 90%. The identification of critical roles of CLEC2 and CLEC5A/TLR2 in platelet-leukocyte interactions will support the development of novel strategies to treat acute viral infection in the future.

(206) Investigating the Role of α2,3 sialylation and poly-LacNac Structures in Cancer Stem Cell Function

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Cancer stem cells (CSCs) are defined as a subpopulation of tumor cells that exhibit self-renewal capacity and the ability to differentiate to other tumor cell populations. The realization that they contribute to resistance to conventional therapies, tumor recurrence and metastasis has heightened the need to understand mechanisms that sustain their function and to develop novel approaches to target them. We are pursuing the hypothesis that the genesis and function of CSCs is intimately associated with specific alterations in their glycome and that these alterations are potential therapeutic targets. In pursuit of this hypothesis, we have used RNA-seq and lectin microarray analysis to compare the glycosyltransferase expression profile and glycomes of breast CSCs and non-CSCs. Comparison of CSCs and non-CSCs from multiple models of breast cancer revealed that CSCs are distinguished from non-CSCs by a marked increase in α2,3 sialylation and poly-LacNac structures. Moreover, the enzymes responsible for these modifications, ST3GAL6 and GCNT2, are enriched in CSCs and expressed preferentially in aggressive breast cancer subtypes. Experiments are in progress to understand the mechanism by which α2,3 sialylation and poly-LacNac contribute to CSC function and to target them in mouse models of breast cancer.

(208) ST6GAL1 -mediated sialylation in intestinal homeostasis and maintenance of microbiome

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We observed that mice unable to express ST6GAL1 are strikingly sensitive to acute radiation injury to the gastro-intestinal tract (GI-ARS), and they have altered fecal microbiome composition. Susceptibility to ionizing radiation and an altered environment to host microbiome are significant health concerns, and our preliminary observations implicate that the intestinal epithelial architecture is intimately affected by the sialyltransferase ST6GAL1. However, ST6GAL1 is generally not expressed in the adult intestinal epithelium, which are largely negative to binding by SNA (a lectin that recognizes the a2,6-sialic acids constructed by ST6GAL1). Therefore, how ST6GAL1 contributes to maintaining the intestinal architectural is not understood.

The intestinal stem cells, a rare cell population residing deep within the crypts of the adult intestinal villi, may be the only intestinal epithelial cells that express ST6GAL1. I will test the hypothesis that presence of active ST6GAL1 protects stem cells from ionizing adiation. Absence of active ST6GAL1 renders the stem cells to radiation destruction as well as promote iologically altered epithelial cells to sustain a microbiome population with decreased *Clostridium, Corprobacillus* and *Aldercreutzia*, and increased *Helicobacter* and *Bilophila*.

(209) MotifFinder, Managing the Glycomics Headache

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With the advent of glycan microarrays, it is now possible to screen glycan-binding proteins quickly and accurately. However, with high throughput screening comes massive amounts of data that is difficult to work with. We present a newly automated version of MotifFinder that can make even the largest glycan database accessible to interdisciplinary researchers. MotifFinder integrates microarray data to assemble motifs from data with varying concentrations and stemming from varying source databases. While traditional methods only identify high-affinity motifs, MotifFinder identifies both strong and weak binders. Additionally, MotifFinder can produce motifs that have variable linkages and residues. Z Biotech has found the automated version of MotifFinder to be intuitive, reliable, and it reproducibly identifies motifs that explain microarray data. As databases grow MotifFinder promises to streamline both glycan database mining and experiment analysis, turning the insurmountable computational analysis into bite-sized biochemical insights into the complex world of glycomics.

(210) Interactions of Tau and Heparin/GAGs

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Tau aggregates into paired helical filaments (PHF) within neurons, a pathological hallmark of Alzheimer's disease. Heparin promotes tau aggregation and recently has been shown to be involved in the cellular uptake of tau aggregates. In current study, we used surface plasmon resonance (SPR) and nuclear magnetic resonance spectroscopy (NMR) to characterize the interaction between tau fragments, K18 and K19, and glycosaminoglycans (GAGs), including heparin, heparin oligosaccharides, chemically modified heparin and other GAGs. Using a heparin-immobilized chip, SPR revealed that tau K18 and K19 bind heparin with K_D of 0.2 mM and 70 mM, respectively. Using SPR competition experiments, *N*-desulfation and 2-*O*-desulfation has no effect on heparin binding to K18, while 6-*O*-desulfation severely reduces binding, suggesting a critical role for 6-*O*-sulfation in tau-heparin interaction. Chondroitin sulfate E and dermatan disulfate can efficiently compete against K18-heparin binding, both containing a 6-*O*-sulfo group. The tau-heparin interaction becomes stronger with longer-chain heparin oligosaccharides. NMR shows largest chemical shift perturbation (CSP) in R2 in tau K18, which is absent in K19, revealing differential binding sites in K18 and K19 to heparin. Dermatan sulfate binding produces minimal CSP while dermatan disulfate, with the additional 6-*O*-sulfo group, induces much larger CSP. 2-*O*-desulfated heparin induces much larger CSP in K18 than 6-*O*-desulfated heparin.

(211) Regulation of Extrinsic Glycosylation in Platelets

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Glycosyltransferases such as the sialyltransferase ST6GAL1 reside in the extracellular spaces, in addition to their canonically recognized locale within the intracellular ER-Golgi secretory apparatus. The existing data point to roles for the extracellular ST6GAL1 in influencing hematopoietic decisions on multiple levels in blood cell development within the marrow, and in modulating inflammation in the periphery. The extracellular milieu is generally devoid of significant levels of sugar donor substrates such as CMP-sialic acid, but previous data from the Lau Laboratory demonstrated that activating platelets release sialic acids that are used for extracellular, or extrinsic sialylation, and posited that the master regulator for extrinsic sialylation is the release of activated sugar donor substrates. However, the origin and nature of the donatable sialic acid within the platelets, and their biosynthetic precursor in the marrow, the megakaryocyte, remain unknown. Disturbed hematopoiesis with highly heterogenous presentation is the defining hallmark of clonal myeloid diseases such as myeloproliferative neoplasms (MPN) and

myelodysplastic syndromes (MDS). A key feature of MPN and MDS are dysplastic megakaryocytes in the marrow and altered circulating platelet numbers and function. I hypothesize that megakaryocytes control marrow extrinsic sialylation in a manner similar to platelets in the periphery. This hypothesis will be tested by biochemically characterizing the activated sugar donor substrates within the platelets, and the glycobiological changes to the thrombocytes and their precursor megakaryocytes in disease.