(369) Synthesis of Inositol Phospholipid as an NKT Cell Modulator

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Entamoeba histolytica membrane has lipopeptidophosphoglycan, which contains inositol phospholipid moieties, EhPIa and EhPIb. The inositol phospholipids are possible natural killer T (NKT) cell ligands and selectively induce IFN-y but not IL-4 in NKT cells, via CD1d dependent manner[1]. Because of these interesting biological activities and the unique structures of EhPIa and EhPIb having characteristic long-chain fatty acids, we synthesized these compounds to clarify the precise biological activities.

We developed new synthetic methods including regioselective phosphorylation reaction of myo-inositol with utilizing chiral phosphatizing reagents such as chiral binaphtol as a chiral auxiliaries and Ni catalyzed *sp3-sp3* cross coupling reaction for the synthesis of the long-chain fatty acids[2]. In order to achieve the total synthesis of these unique inositol phospholipids, we adopted a new protecting group strategy utilizing Allyl and Alloc groups as the permanent protecting groups for the hydroxyl groups in the inositol. These protecting groups were cleaved at the final step of the total synthesis with Ru complex[3] highly effectively. Based on these newly developed methods, we succeeded in the first total syntheses of EhPIa and EhPIb.

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(370) Human proteome microarray-based substrate profiling of ppGalNAc-T2 reveals intracellular proteins O-GalNAc modification

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Protein O-GalNAc glycosylation is initialized by members of the UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase (ppGalNAc-T) family. The human ppGalNAc-T family has 20 members where 15 isoforms have UDP-GalNAc transfer activity and each member displays tissue-specific expression and substrate-specific activities. To decipher the substrate specificity of ppGalNAc-Ts, we developed a strategy for global identification of the protein substrate of ppGalNAc-T by combing the high-throughput analysis capability of protein microarrays and the high specificity of click chemistry. Using this strategy, we systematically screened potential substrates of ppGalNAc-T2 and identified about 300 candidates as possible O-GalNAc glycosylated. All of them, in addition to membrane-associated proteins, interestingly, intracellular proteins such as transcriptional factors and cytoskeleton-related proteins were identified by O-GalNAc glycosylation. Several selected intracellular proteins of them were validated by lectin blotting and analysis. Enzyme reaction analysis (such as dC1GalT1, O-Glycosidase) and lectin blot suggested that they could be O-GalNAc glycosylated in cells. The protein substrates of ppGalNAc-T2 identified in this study could serve as a valuable source for future functional studies. The microarray-based strategy is general and suitable for protein substrate profiling of other ppGalNAc-Ts and should be helpful for systematically understanding the function of ppGalNAc-T.

(371) Study on oligosaccharides delivery system with supercritical carbon dioxide

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We have studied aiming to develop the system to carry the oligosaccharides as the medicine to the focus in the inside of the body appropriately and surely. It is a heavy-duty function to pass on information outside the cell to the cell for the multicellular organism, and is called the signaling. When an appropriate oligosaccharides is insufficient, it causes the sickness and the functional lesion because the oligosaccharides is used for the signaling. When the oligosaccharides is insufficient, it is necessary to carry to the place for which the oligosaccharides needed in the inside of the body is needed. We involved the oligosaccharides to the career and developed the method of doing delivery to the diseased part. The substance that composed the cell membrane of the living body where the rejection caused by the human body was not caused was selected as a material of the career. An artificial cell membrane was made by making the Dipalmitoylphosphatidylcholine and cholesterol that was the element of the cell membrane a material and using supercritical of carbon dioxide. An artificial cell membrane was made a system that was able to control the size. Moreover, the method of controlling the shear thinning of the career by the magnetic field that had been set up outside of the people body to be established to an arbitrary place in the inside of the body of an artificial cell membrane that involved the oligosaccharides was developed. The metallic oxide that reacted to the magnetic field was involved in an artificial cell membrane. The situation in which the career injected with from the vein moved in the blood vessel and it stayed only in the vicinity of the diseased part was simulated. It is thought that basic data the oligosaccharides delivery system that uses an artificial cell membrane is designed was able to be obtained from the above-mentioned result.

(372) Glycolipid biosurfactant enhanced biodegradation of aromatic

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Microorganisms have the exceptional ability to exploit toxic substances for growth, which is the basis of bioremediation. However, bacterial utilization of persistent toxic substances is limited by poor bioavailability due to their high hydrophobicity and tight adsorption on soil particles. *Burkholderia* sp. C3, a bacterial species isolated from polluted soil, can degrade polycyclic aromatic hydrocarbons (PAHs)

such as dibenzothiophene (DBT) and pesticides. Chemical analysis and bioassays revealed that glycerol induced C3 to secrete rhamnolipids which enhanced biodegradation of the aromatics and lessened the toxicity to allow bacterial growth. Rhamnolipids are non-toxic and biodegradable glycolipid biosurfactants. Rhamnolipids secreted by C3 included mono- and dirhamnolipid congeners. The relevant proteins profiled and the rhamnolipid biosynthetic genes (i.e., *rhl* genes) identified allow further understanding of relationships among PAHs metabolism, cell growth and biosynthesis of rhamnolipid. Rhamnolipids probably enhance bioavailability of the aromatics by increasing water solubility and membrane transport into C3 cells. Biodegradation of DBT occurs through Kodama pathway, but not Desulfurization pathway. C3 contains 7 proteins involved in this pathway, including 2 dioxygenases and 1 monooxygenase. These proteins were identified in C3 cells when DBT and a mixture of glycerol and DBT were the substrates. However, 17 groups of ABC-type transporters were detected when glycerol was present, in contrast to only 4 groups when DBT alone was used as a substrate. Four phasin proteins involved in lipid biosynthesis were also observed in the former condition, in comparison to 2 in the latter condition. This study demonstrated connections and networks between phasin up-regulation, ABC-type transporter up-regulation, rhamnolipid production and DBT biodegradation. The results provided insights into bacterial adaptation to overcome toxic stress conditions and might lead us to the improvement of bioremediation technologies.

(373) Antibody free genome-wide chemical mapping suggests O-GlcNAc confers polycomb responsive element-independent silencing

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ß-O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT) is one of the set of Polycomb group proteins (PcGs), which are involved in silencing homeotic (Hox) genes during development. Here we describe an antibody-free chemoselective tagging method that provides a robust alternative to standard chromatin immunoprecipitation (ChIP). Because this strategy does not depend on the use of antibodies it permits unbiased mapping of genomic loci enriched in O-GlcNAc. Using this genome wide approach we find O-GlcNAc is abundant at loci other than Polycomb Responsive Elements (PREs). We further demonstrate that loss of O-GlcNAc enhances expression of genes at these loci - suggesting a role for O-GlcNAc in silencing that is independent of PREs.

(374) A proteoglycan system controlling growth factor activity, stem cell fate and neurogenesis in the adult brain

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New neurons and glia are continuously produced in the adult mammalian brain. The new neural cells result from neural stem cell proliferation and differentiation in restricted neurogenic zones: the subventricular zone (SVZ) of the lateral ventricle, the rostral migratory stream, the sub-callosum zone and the dentate gyrus sub-granular layer. Numerous growth factors operate in the neurogenic environment and influence neural stem cell fate. However, the precise mechanisms underlying neurogenesis, and the reason for which neurogenesis occurs only in confined zones, are unknown. We have shown high expression of N-sulfated heparan sulfate proteoglycans (NS-HSPG) in all adult mouse neurogenic zones. NS-HSPG immunoreactivity delineates a continuum connecting the neurogenic zones to each other and to the meninges. Thus, on the contrary to the current belief, a single neurogenic system rather than different zones, exists. Besides meninges, the NS-HSPG-expressing system consists of specialized extracellular matrix structures named fractones and of the adventitia (meningeal layer) of arterioles. In the most neurogenic zone, the SVZ of the lateral ventricle, we have demonstrated that NS-HSPG associated with fractones bind and activate fibroblast growth factor-2 (FGF-2), bone morphogenetic protein-4 (BMP-4) and BMP-7, which in turn regulate neural stem cell proliferation. HSPG act as extracellular matrix receptors promoting the interactions between growth factors and its cognate cell-surface receptors. High expression of gap junctions along the NS-HSPG-expressing system in the neurogenic zones, and in the meninges between fibroblasts and between macrophages, suggests that an intercellular communication network may coordinate NS-HSPG expression. We report experiments supporting the view that the NS-HSPG immunoreactive meningeal extension leading to the lateral ventricle SVZ (falx cerebri plus sub-hippocampal meninge) is a long distance communication network. Showing that this NS-HSPG network may function over long distance, we demonstrate that 6OHDA fibroblast ablation in this meninge at the brain surface specifically increases cell proliferation in the SVZ neurogenic zone (i.e. millimeters away). Taken together, our results support the view that meninges and fractones form a integrated connective tissue system that operates with HSPG to regulate growth factor activity and control neural stem cell fate in the adult brain.

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(375) Mouse Siglec-E Engages Hyaluronan and Modulates Group A Streptococcal Infection

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Sialic acid-binding immunoglobulin-like lectins (Siglecs) are inhibitory receptors expressed on leukocytes, and are known to modulate innate and adaptive immune functions. It is well established that sialic acids can function as self-associated molecular patterns (SAMPs) to engage Siglecs and dampen cell activation. Recently, we discovered a second SAMP system in which human Siglec-9 binds to high molecular weight hyaluronan (HMW-HA) and dampens neutrophil extracellular trap (NET) formation, oxidative burst and apoptosis. In keeping with this, group A *Streptococcus* (GAS) uses its HMW-HA capsule as molecular mimicry to evade human neutrophil killing by engaging hSiglec-9 (Secundino et al., submitted). We have now discovered that mouse Siglec-E (mSiglec-E), the functional ortholog of Siglec-9 (hSiglec-9), also binds HMW-HA. Our subsequent studies were aimed at determining whether mSiglec-E had similar effects *in vitro* and *in vivo* on GAS pathogenesis.

Our results show a defect in immune clearance of GAS in whole blood, and neutrophil killing assays ex vivo in wild type mice compared to Siglec-E-deficient mice. This suggests that deletion of Siglec-E resets the immune system to fast activation, and fast elimination of the bacteria. However, deletion of an inhibitory receptor may also affect counterbalancing of the immune system and cause more harm to the host. In order to test this hypothesis, we infected wild type and Siglec-E-deficient mice subcutaneously with GAS. In initial studies, lesion sizes for Siglec-E-deficient mice were significantly larger and showed a defect in lesion healing, compared to wild type mice. Future studies are warranted to investigate the role of Siglec-E in a sepsis model, and the importance of immune system regulation during GAS infection.

(376) Mechanistic roles for glycoproteins in intestinal cell intoxication by cholera toxin

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Exotoxins secreted from bacteria typically bind glycoconjugates on the surface of host cells, then enter host cells and transduce signals. However, mechanistic steps in host cell intoxication are poorly defined. To identify glycoconjugates recognized by exotoxins, we developed a photocrosslinking strategy that relies on metabolic incorporation of diazirine-modified sialic acid (SiaDAz) into host cell glycoconjugates. UV activation of the diazirine photocrosslinker results in covalent crosslinking between an exotoxin and its sialylated binding partners. Using this method, we showed that cholera toxin crosslinks to glycoproteins in a human intestinal epithelial cell line. Culturing cells with alpha-benzyl-GalNAc, which interferes with mucin-type O-linked glycosylation, resulted in altered mobility of the covalent complexes between cholera toxin and host glycoproteins, suggesting that the toxin recognizes O-linked glycoproteins on human intestinal epithelial cells. Next, we performed functional assays to determine the functional role of glycoproteins in host cell entry and intoxication. Surprisingly, cholera toxin-induced cAMP accumulation was inhibited by not only alpha-benzyl-GalNAc, but also kifunensine (an inhibitor of N-linked glycosylation) and NB-DGJ (an inhibitor of ganglioside synthesis). We also performed binding assays to assess which class of glycoconjugates is the dominant binding partner for cholera toxin. We discovered that O-linked glycoproteins are the main toxin binding partners in a human intestinal epithelial cell line, while gangliosides contribute most in a mouse epithelial cell line. We will also report the effect of glycosylation inhibitors on internalization and localization of cholera toxin.

We investigated the identity of glycoproteins recognized by cholera toxin using both mass spectrometry and a candidate approach. Mass spectrometry analyses of the cholera toxin crosslinked complex identified multiple proteins, including CEACAM5. The interaction between CEACAM5 and cholera toxin was confirmed by immunoprecipitation analysis, but, interestingly, depends on N-linked glycosylation. We speculated that cholera toxin also recognizes additional O-linked glycoproteins. Because mucins are abundant O-linked glycoproteins in the intestinal epithelial, additional experiments are underway to assess the ability of cholera toxin to bind mucins. In summary, we report that cholera toxin binds different glycoconjugates in human and mouse cell lines, and describe progress toward defining the roles of specific cholera toxin-binding glycoproteins in host cell intoxication.

(377) Modulation of Immune Responses via TLR4/MD-2 with Synthetic Isoprenoids

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Innate immune receptors are activated by particular components common to microbes, and also by some compounds with different types of backbone structures, including endogenous compounds. The complex of toll-like receptor 4 (TLR4) and myeloid differentiation factor 2 (MD-2) is known as the receptor of lipopolysaccharide (LPS) from Gram-negative bacteria, and the receptor protein recognizes the ligand at the lipophilic terminal moiety, lipid A.

As a TLR4/MD-2 ligand that has different backbone from lipid A, we have recently found a lipid A-mimic compound, FNC-R-01, from a high-throughput screening. FNC-R-01 is an analog of funiculosin isolated from a soil bacterium, *Penicillium funiculosum*. We then elucidated the chemical structure of FNC-R-01 and established the preparation method of FNC-R-01 from funiculosin. Molecular modeling studies of FNC-R-01 and its derivatives estimated the binding conformation FNC-R-01 and funiculosin with TLR4/MD-2. Other derivatives of funiculosin and FNC-R-01 were also designed by the molecular modeling. Some of the synthesized derivatives showed immunomodulatory activities and might be useful as immunostimulatnts/adjuvants.

(378) High throughput screening and synthetic study of Fut8 inhibitors

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Fucosyltransferase8 (Fut8) is an enzyme that transfers a fucose from GDP-fucose to the reducing end N-acetylglucosamine of the N-glycan to form core fucose. Core fucose has various biological activities. For example, core fucose controls Antibody-Dependent Cellular Cytotoxicity (ADCC) of immunoglobulin IgG. α-Fetoprotein (AFP) containing core fucose has been used as a biomarker of hepatic cancer. We aimed to develop the Fut8 inhibitor that works in *in vivo* system to investigate the biological function of core fucose. This study includes two approaches, high throughput screening (HTS) and chemical synthesis.

In the HTS approach, we first constructed the assay system to measure the Fut8 activity. GDP, which is produced from GDP-fucose in Fut8 enzyme reaction, was detected by fluorescence polarization using anti-GDP antibody and fluorescent labeled GDP. We tested 33000 compounds from Osaka University's chemical library for the HTS. The inhibitory activity of the hit compounds were

then assessed by using HPLC system. The hit compounds obtained after the second screening showed 10-100 μM inhibitory activity. It was noteworthy that five of them had the common pharmacophore. We are investigating the inhibition mechanism of these inhibitors and the structural expansion to obtain more potent inhibitors.

In the synthetic approach, we designed GDP-fucose mimics as Fut8 inhibitors. To realize the efficient divergent synthesis of inhibitor candidates, the appropriate mimics of diphosphate moiety of GDP-fucose were constructed by the reactions between alkyne and sulfonyl azide. First, the model reactions of alkyne with sulfony azide were carried out under several conditions to find the good conditions for alkyne-sulfony azide coupling. Then, we prepared alkyne containing fucose unit and sulfonyl azide containing guanine unit, and the coupling reaction successfully afforded the several inhibitor candidates.

(379) Sialylated Glycoconjuate-Specific Binding Lectin from the Helicium erinaceum

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Mushroom lectins harboring carbohydrate binding specificity are a powerful tool to detect sialoglycoconjugate with α(2,3)-, α(2,6)-, and α(2,8)-likages. However limited numbers of sialic acid-specific binding lectins are available to detect linkage of glycoconjugates. In this study, a sialic acid-specific binding lectin was identified and characterized from the fruiting body extract of a mushroom *Helicium erinaceum*. The sialic acid binding lectin was purified by a combination of ion-exchange column, an immobilized fetuin column and gel filtration chromatography. SDS-PAGE and N-terminal amino acid sequencing indicated that the native lectin, designed *H. erinaceum* lectin (HEL), is an identical form with a molecular weight of approximate 15 kDa. Isoelectric focusing of the lectin showed bands near pI 5.4. Agglutination assay displayed HEL was more effective toward porcine's erythrocyte rather than other animals red blood cells. When the HEL was analyzed with glycan array, it was observed that the lectin binds to Neu5Ac, Neu5Gc and other sialic acid derivatives. Furthermore, HEL bound to α(2,3)-sialylated fetuin and not to asialofetuin. Thus, HEL could be a promising tool for detection of linkage-specific sialic acid in glycoconjugates.

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(380) EtpA adhesin engages intestinal mucins to facilitate enterotoxigenic E. Coli host colonization and pathogenesis

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Enterotoxigenic *E. coli* (ETEC) is an important cause of diarrhea, particularly in young children under 5 years of age, accounting for hundreds of thousands of deaths yearly in the developing world. Colonization of the host small intestine is a critical virulence feature as engagement of mucosal surfaces facilitates effective delivery of ETEC enterotoxins to epithelial receptors, ultimately promoting a net efflux of water into the intestinal lumen and ensuing diarrhea. Understanding host-pathogen interactions essential to effective colonization can provide key insights into relevant ETEC vaccine design. EtpA, a secreted glycoprotein adhesin, plays an important role in ETEC intestinal colonization. EtpA interacts with conserved regions of flagellin (FliC) exposed at tips of the flagella and enables bacteria adhere to the host surface by acting as a molecular bridge. Here we demonstrate that EtpA binds to host intestinal mucins. In vitro protein interaction studies involving far western, co-immunoprecipitation and/or biolayer interferometry techniques demonstrated that EtpA interacts with the gel-forming mucin MUC2 as well as with epithelial transmembrane MUC3 molecules. Oxidation of MUC2 but not of EtpA with sodium metaperiodate abolished this interaction. Consistent with this observation, EtpA exogenously added to intestinal sections from WT mice co-localized with Muc2; however this interaction was highly abrogated in sections from mice lacking Core 3 O-glycans suggesting specific recognition of mucin O-glycans by EtpA. RNAi-mediated depletion of transmembrane MUC3 mucin *in vitro* significantly reduced ETEC epithelial cell adhesion and heat-labile toxin delivery. Further, *in vivo* studies showed rapid clearance of *etpA* mutant bacteria by the infected mice compared to wt ETEC strain H10407. These studies further highlight the importance of EtpA in pathogenesis and are providing important molecular interaction data to exploit in rational approaches to vaccine design.

(381) Synthesis and Evaluation of Monophosphoryl Lipid A Derivatives as Fully Synthetic Self-Adjuvanting Glycoconjugate Cancer Vaccine Carriers

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Fully synthetic carbohydrate-based cancer vaccine is an attractive concept, while an important topic in the area is to develop proper vaccine carriers that can improve the immunogenicity and other immunological properties of tumor-associated carbohydrate antigens (TACAs). In this context, four monophosphoryl derivatives of *Neisseria meningitidis* lipid A were synthesized via a highly convergent and effective strategy and evaluated as vaccine carriers and adjuvants. The conjugates of these monophosphoryl lipid A (MPLA) derivatives with a modified form of the sTn antigen were found to elicit high titers of antigen-specific IgG antibodies, indicating T cell-dependent immune response, in the absence of an external adjuvant. It was concluded that MPLA's could be utilized as potent vaccine carriers and built-in adjuvants to create fully synthetic self-adjuvanting carbohydratebased cancer vaccines. The lipid composition and structure of MPLA were shown to have a significant influence on its immunological activity, and among the MPLA's examined, natural *N. meningitidis* MPLA exhibited the most promising properties. Moreover, Titermax Gold, a conventional vaccine adjuvant, was revealed to inhibit, rather than promote, the immunological activity of MPLA conjugates, maybe via interacting with MPLA text goes here.

(382) Reishi Polysaccharides-induced Antibodies Recognize Tumor- Associated Carbohydrate Epitopes

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Detailed evaluation of Reishi (Ganoderma lucidum or Ling-Zhi, a medicinal fungus) is challenging. While Reishi has been used for thousands years by people all over the world to treat disease and promote health, the exact mechanism of action is still not well understood. The saccharide portion of glycoproteins and glycolipids of Reishi contains a wide variety of immunogenic epitopes that may induce predominantly antibody responses. Therefore, the carbohydrate moieties of Reishi are thought to be important antigenic determinants. By applying carbohydrate microarray analysis, we show here for the first time that antibodies to a cancer associated carbohydrate epitope, Globo H, are induced in mice after immunization with a fucose-containing fraction of Reishi (FFOR), thereby exerting anticancer activity. This is a previously unrecognized but important mechanism. A more exciting finding is that immunostaining of a FFOR with the known anti-Globo H monoclonal antibodies, VK9 and MBr-1, suggests that a tumor-associated carbohydrate epitope "Globo H-like" structure existing in FFOR. We anticipate that our results would lead to the development of Reishi polysaccharides as cancer vaccines capable of inducing epitope-specific anti-cancer antibodies and contribute our understanding of FFOR-mediated anti-cancer activity. Furthermore, we demonstrate the utility of carbohydrate microarrays for evaluating immune responses to large, complex immunogens such as herbal medicine and the ability to detect previously unrecognized epitopes.

(383) Lectin Nucleotide Phosphohydrolases (LNPs): a Family of Lectins that May Function as Co-Receptors or Modulators of Oligosaccharide-Signaling Events in Plants

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Previous biochemical studies from our laboratory identified a novel lectin in the roots of the legume, *Dolichos biflorus*, that binds to the lipochitooligosaccharide Nod factor produced by rhizobia that can nodulate the roots of this plant. Characterization of this lectin showed that it is an apyrase with nucleotide phosphohydrolase activity. This Lectin Nucleotide Phosphohydrolase (LNP[1]) is a peripheral membrane protein localized on the surface of root hairs in the nodulation zone. Recent transgenic studies (1) from our laboratory have established that LNP1 functions, possibly as a co-receptor or modulater, in the initiation of the Nod factor-stimulated rhizobium-legume nitrogen-fixing symbiosis. This LNP (or a closely related homolog of it) also plays a similar role in the initiation of the Myc factor-stimulated fungal symbiosis that occurs in the roots of about 80% of plant species and results in the facilitation of nutrient uptake.

Using a combination of biochemical, cellular and molecular biological techniques we have now identified at least three other proteins in *Dolichos biflorus* that are probable LNPs. The differential expression of these proteins, both spatially and temporally, in the plant suggests that this family of proteins may have arisen by gene duplication and subsequent divergence to function in other oligosaccharide signaling events in the plant.

(Supported by NIH Grant GM21882 and by Ceres, Inc.)

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(384) Interaction of alpha2,6-linked sialic acids with siglec-2 modulates the adhesion of hepatocarcinoma cells to lymph nodes through FAK signaling pathway

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The alterations of cell surface sialylation play a key role in tumor metastasis. Enhanced α2,6-sialylation on N-glycans results from overexpression of the Golgi enzyme β-galactoside: α2,6-sialyltransferase (ST6Gal-I). Hca-F and Hca-P cells are murine hepatocarcinoma cell lines with high and low potential of lymphatic metastasis, respectively. Our previous study revealed that ST6Gal-I was involved in the adhesion of Hca-F cells to fibronectin. However, the roles of sialic acids in the adhesion of Hca-F cells to lymph nodes still remain poorly understood.

In this study, we observed that expression levels of α2,6-linked sialic acids on Hca-F cells were higher compare to Hca-P cells. Knockdown of ST6Gal-I by shRNA transfection decreased the expression of α2,6-linked sialic acids, and inhibited the adhesion of Hca-F cells to lymph nodes. Hca-F cells exhibited the highest binding capability to siglec-2 rather than to other siglecs (siglec-1, 2, 3, 5), which were expressed in lymph nodes. More importantly, cell adhesive capability to lymph nodes was reduced gradually in the presence of anti-siglec-2 antibody, and the inhibition percentage was dependent on the concentration of the antibody. ST6Gal-I knockdown inhibited the phosphorylated levels of FAK and paxillin when cells were treated with siglec-2. Taken together, these results suggest that interaction of α2,6-linked sialic acids with siglec-2 might modulate the adhesion of hepatocarcinoma cells to lymph nodes through FAK signaling pathway, suggesting a new mechanism of tumor lymphatic metastasis.

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(385) Keeping Track of Sperm Glycans

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The roles of glycans during fertilization and early embryonic events are an established and well-studied aspect of glycobiology. However, there is a substantial deficit in our understanding of the glycobiology of spermatogenesis, epididymal sperm maturation and sperm function during pre-fertilization events. Glycans are the most highly represented molecules at the cell surface and they may also be one of the most informative class of signaling molecules known to date. The complex interplay between N-Glycans, glycosamioglycans and glycolipds leads to an incredible number of possible signaling motifs that could regulate sperm maturation and function and, as a result, fertility. The purpose of this poster is to summarize the current understanding of glycan dependent processes during spermatogenesis, sperm maturation and sperm function in the female reproductive tract. In addition, the review will highlight specific areas of glycobiology that have not been extensively studied in the context of sperm development and function.

(386) Expression and Functional Characterization of Siglec-9 in Mice

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Neutrophilic inflammation is a major component in the pathogenesis of acute and chronic pulmonary diseases such as acute airway infections and chronic obstructive pulmonary disease (COPD). Effective therapies for neutrophil-dominated inflammatory lung disease are still lacking. Sialic acid binding Ig-like lectin receptors (Siglecs) mediate sialic acid-dependent interactions with ligands. Siglec-9 and its murine ortholog, Siglec-E, are expressed mainly on neutrophils and macrophages. Siglec-E has been shown as a negative regulator of host inflammatory responses through induction of cell death as Siglec-E knockout (KO) mice mounted enhanced inflammation. Although Siglec-9 has been shown to regulate immune responses in vitro, study of Sgilec-9 in the context of inflammation in vivo is proven difficult. We sought to generate genetically modified mice that carry and can express human Siglec-9 in inflammatory cells and to study its regulatory function and mechanisms in inflammation models. Through homologous recombination, Siglec-9 knock-in (KI) mice were generated by replacing the exons of the Siglec-E gene with Siglec-9 cDNA downstream of the Siglec-E promoter. Homozygous KI mice carry the Siglec-9 cDNA but not the Siglec-E gene. Without stimulation, Siglec-9 KI animals are phenotypically normal. Wild type, Siglec-9 KI and Siglec-E KO mice were then compared under inflammation conditions: lipopolysaccharide (LPS), (1 Qg, i.t., for 24 hrs) or porcine pancreatic elastase (1 Unit, i.t., for 48 hrs). Bronchoalveolar lavage was performed and cell counts and differential were determined. When challenged, Siglec-9 KI mice showed neutrophil-dominated inflammatory responses in the airway similar to those in wild type mice. In contrast, Siglec-E KO mice showed significantly enhanced inflammation in the airway, whereas the cell differential counts were similar in all three groups. Siglec-9 mRNA, not Siglec-E mRNA, can be seen in inflammatory cells isolated from Siglec-9 KI mice. These cells were also able to bind to synthesized Siglec-9 ligand-decorated liposomes. These data not only confirm that Siglec-E plays a critical role in regulating airway inflammation but also show that Siglec-9 can be expressed in mice, is able to bind to its ligand, and be able to control inflammation in vivo. These mice are a useful tool for studying human Siglec-9 in various disease models.

(387) Glycoengineered Outer Membrane Vesicles Displaying O-Polysaccharide Antigens Elicit Protective Antibodies Against Francisella tularensis

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The O-polysaccharide antigen (O-antigen) component of lipopolysaccharide on the surface of Gram-negative bacteria is both a virulence factor and a B-cell antigen. Conjugate vaccines used against multiple pathogens take advantage of the fact that O-antigens can elicit antibodies that often confer protection against infection. However, conventional methods for natural extraction or chemical synthesis of O-antigens for production of these conjugate vaccines are technically demanding, inefficient, and expensive. Here, we describe a scalable glycoengineering technique wherein recombinant O-antigen biosynthesis is coordinated with vesiculation in lab strains of *Escherichia coli* to produce acellular outer membrane vesicles (OMVs) decorated with pathogen-specific O-antigens on their surfaces. Because genes required for the biosynthesis of a given O-antigen are usually located in a distinct cluster, they can be easily amplified and transferred into E. coli for efficient, large-scale production. Moreover, the use of OMVs greatly simplifies glycoconjugate assembly and purification and results in a self-adjuvanting O-antigen delivery system. Immunization of mice with engineered OMVs displaying O-antigens from *Francisella tularensis* subsp. tularensis Schu S4 protected against intraperitoneal challenge with *F. tularensis* subsp. tularensis Schu S4, demonstrating the vaccination potential of our glycoconjugate vesicles.

(388) Identification of Arabidopsis α1,3-fucosidase acting on plant complex type N-glycans and degradation pathway of plant N-glycans

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Plant N-glycans are roughly classified into high-mannose and plant complex type. Their typical N-glycans, M5A [Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc] and M3FX [Manα1-6(Manα1-3)(Xylβ1-2)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc], respectively, are found as major glycans in Arabidopsis. The degradation pathway of high-mannose type N-glycans has been revealed by substrate specificity analysis of plant specific glycosidases, endo-β-mannosidase and vacuolar α-mannosidase. These glycosidases cooperatively hydrolyze M5A in vacuole. However, the degradation pathway of M3FX, typical complex type N-glycan in plant, has remained to be clarified. In this pathway, four glycosidases (endo-β-mannosidase, vacuolar α-mannosidase, β1,2-xylosidase, and α1,3-fucosidase) are supposed to be involved in. Among them, only the substrate specificity of α1,3-fucosidase has not been determined. In this study, we attempted to identify Arabidopsis α1,3-fucosidase acting on the Fucα1-3GlcNAc linkage in the M3FX and analyze its substrate specificity to clarify the degradation pathway of plant complex type N-glycans.

As a candidate, we chose the Arabidopsis α1,3/4-fucosidase, which has been reported not to act on a plant complex type N-glycan, Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-3)GlcNAc. This protein has also reported to be expressed in vacuole. This protein was expressed in Pichia pastoris as a soluble protein. When this recombinant protein was assayed against several truncated-plant complex type N-glycans, it revealed that the protein hydrolyzed only the Fucα1-3GlcNAc linkage of GlcNAcβ1-4(Fucα1-3)GlcNAc (GN2F). This substrate specificity is compatible to those of other glycosidases acting on plant complex type N-glycans, and the degradation pathway of M3FX in vacuole has been emerged. In the α1,3-fucosidase deficient mutant, GN2F, a substrate of α1,3-fucosidase, was considerably accumulated, supporting that this glycosidase acts on plant complex type N-glycans. The difference of phenotypes between the wild type and the mutant plants has not been detected in as far as we observed.

(389) The use of EGALC reveals the presence of a novel ether-linked phytol-containing digalactosylglycerolipid in the marine green alga, Ulva pertusa

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Galactosylglycerolipids (GGLs) and chlorophyll are characteristic components of chloroplast in photosynthetic organisms. Although chlorophyll is anchored to the thylakoid membrane by phytol (tetramethylhexadecenol), this isoprenoid alcohol has never been found as a constituent of GGLs. We here describe a novel GGL, in which phytol is linked to the glycerol backbone via an ether linkage (Ishibashi et al, 2014). This unique GGL was identified as an Alkaline-resistant and Endogalactosylceramidase (EGALC)-sensitive GlycoLipid (AEGL) in the marine green alga, Ulva pertusa. EGALC is an enzyme that is capable of cleaving the β-glycosidic linkage between R-Galα/β1-6Gal and the lipid moiety, thereby releasing intact galactooligosaccharide from glycolipids. The specificity of EGALC is very high for the sugar moiety, but relatively wide for the lipid moiety, i.e., it hydrolyzes GGLs as well as glycosphingolipids (GSLs). EGALC also catalyzes transglycosylation reaction, in which the intact sugar chains are transferred from GSLs/GGLs to the primary hydroxyl group of various 1-alkanols to generate neoglycoconjugates. We previously developed a sensitive and reliable method to detect GSLs/GGLs that share the R-Gal**α**/**β**1-6Gal**β**1- structure, using the transglycosylation reaction of EGALC. The use of EGALC revealed that AEGLs were ubiquitously distributed in not only green, but also red and brown marine algae; however, they were rarely detected in terrestrial plants, eukaryotic phytoplankton, or cyanobacteria. The structure of U. pertusa AEGL was determined following its purification to 1-O-phytyl-3-O-Gal**α**1-6Gal**β**1-sn-glycerol by mass spectrometric and nuclear magnetic resonance analyses. To the best of our knowledge, this is the first study to identify a phytol-containing GGL that may be present in the thylakoid membrane of chloroplasts in marine algae. Since GGLs have been shown to possess important roles in the organization and stabilization of the thylakoid membrane, the novel GGL, AEGL, could have a specific role in photosynthesis in marine algae.

Ishibashi Y, Nagamatsu Y, Miyamoto T, Matsunaga N, Okino N, Yamaguchi K, Ito M. 2014. A novel ether-linked phytol-containing digalactosylglycerolipid in the marine green alga, Ulva pertusa. Biochem. *Biophys. Res. Commun.* (Articles in press)

(390) Synthesis of OAADPR Analogs and their Inhibitory Activities to Human Sirtuin Homolog SIRT1

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OAADPR (O-Acetyl-ADP-Ribose) is a metabolite product of nicotinamide adenine dinucleotide (NAD+)-dependent enzymes, histone deacetylases, named as Sirtuins as well, which play important roles in gene silencing, metabolism regulation, lifespan extension and other cellular processes. More and more evidence suggested that OAADPR is a potential signal molecule with novel biological activities or acts as substrate of related enzymes to regulate their downstream processes. Here we described to synthesize OAAPDR analogs containing a tertiary alcohol at the 2-position of ribose to mimic the tetrahedral intermediate in the acetyl ester hydrolysis. Inhibitory assay indicated that these analogs were uM range inhibitors of human sirtuin homolog SIRT1.

(391) O-glycosylation is essential for nuclear pore integrity and maintenance of the pore selectivity filter

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O-glycosylation of the nuclear pore complex (NPC) by O-linked N-acetylglucosamine (O-GlcNAc) is conserved within metazoans. Many nucleoporins (Nups) comprising the NPC are constitutively O-GlcNAcylated but the functional role of this modification remains enigmatic. We show that O-GlcNAc is critical for maintenance of the nuclear pore selectivity filter in both dividing and post-mitotic cells. Loss of O-GlcNAc, induced by either chemical inhibition of O-GlcNAc transferase (OGT) or deletion of the gene encoding OGT, leads to decreased cellular levels of natively O-GlcNAc modified Nups. Loss of O-GlcNAc enables increased ubiquitination of these Nups and their consequent proteosomal degradation. The decreased half-life of deglycosylated Nups manifests in the gradual loss of these Nups from the NPC and associated malfunction of the selective permeability barrier. These findings define a critical role of O-GlcNAc modification of the NPC being to maintain its composition and function. The results implicate NPC glycosylation as a physiological regulator of NPC function, and reveal the role of conserved stoichiometric glycosylation of the NPC among all metazoans.

(392) Human consumption of yeast containing foods has driven adaptations in the gut microbiota

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The architecture of the human distal gut microbiota is sculpted by the complex carbohydrates delivered in the diet. Yeasts, which are among the earliest domesticated microorganisms and have been a component of the human diet for at least 7000 years, possess an elaborate cell wall **α**-mannan. The influence of yeast mannan on the ecology of the microbiota, however, is unknown. Significantly, *Bacteroides thetaiotaomicron* (*Bt*), a dominant and widespread member of the human large bowel microbial community, has a genome that encodes an extensive repertoire of enzymes capable of hydrolyzing **α**-mannosidic linkages. Furthermore, distinct polysaccharide utilization loci (PULs) orchestrate the differential degradation of yeast **α**-mannan and high mannose mammalian N-glycans (HMNGs) in spite of their structural similarities. We show here using transcriptional analysis, enzyme specificity and cellular localization that yeast **α**-mannan is a viable food source for *Bt*. The deconstruction of yeast mannan was explored by characterizing the key enzymes encoded by the three mannan PULs of Bt. We propose a model whereby limited cleavage of the Ð-mannan backbone by surface GH76 endo-**α**1,6-mannanases generates large oligosaccharides that are depolymerized to mannose in the periplasm through the synergistic action of a GH38 **α**-mannosidase and an array of phosphatases, endo-**α**1,6-mannanases and GH125 exo-**α**1,6-mannosidases. The surface and periplasmic endo-mannanases possess specificities driving cleavage of the mannan backbone into large and small products, respectively, and the unique substrate-promiscuous GH38 **α**-mannosidase, BT3774, releases side-chain sugars directly linked to the backbone of the polysaccharide and exposes the phosphate groups to the monoester phosphatases. While synergistic interactions between members of the microbiota contribute to the utilization of highly accessible glycans, the metabolism of yeast mannan by *Bt* presents a 'selfish' model for the catabolism of this recalcitrant polysaccharide. This report shows how a cohort of highly successful members of the microbiota have evolved to consume sterically-restricted glycans presented on the surface of yeasts, an adaptation to the domestication of the microorganism and its incorporation into the civilized human diet.

(393) Heparan Sulphate Degradation by the Human Gut Symbiont Bacteroides thetaiotaomicron

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Bacteroides thetaiotaomicron (*Bt*) is a human gut symbiont which, primarily utilizes host dietary glycans that prove recalcitrant to the host degradative glycome. Some such glycans are the gylcosaminoglycans (GAGs) heparin/heparan sulphate. These polysaccharides are found ubiquitously on the surface of all mammalian cells. This may suggest that this is a host glycan which *Bt* can utilise but this is unlikely as the polysaccharide utilization locus (PUL), a discrete genomic region conferring *Bt's* ability to degrade heparin/heparan sulphate, is not switched on when mice are fed a simple sugar diet, suggesting that the source of heparin/heparan sulphate which *Bt* targets is derived from the human diet with the likely source being from ingested animal tissue.

Presented here is a detailed kinetic study of the enzymes which are encoded by this PUL. This includes four lyases belonging to polysaccharide lyase (PL) families 12,13 and 15, as well as a glycoside hydrolase 88 and two GAG specific sulphatases. The work reveals an interplay between the 3 predicted periplasmic lyases with differing sulphation pattern requirements and processivity. The predicted surface lyase has a much slower catalytic rate and large binding cleft suggesting little degradation is performed on the surface of the bacteria. The work presented also describes a brand new activity for PL15 and provides the first ligand bound structures of a two heparin/heparan sulphatases.

(394) Lack of Fc Glycosylation Results in Susceptibility to Selective Free Thiol Formation in the CH2 Domain of an E. coli-expressed Recombinant Monoclonal IgG1 Antibody

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Production of recombinant monoclonal antibodies in non-mammalian expression systems offers new opportunities but also new challenges. Variability of the total free thiol content was observed during the development and production of a therapeutic, recombinant, monoclonal IgG1 antibody expressed in the E. coli system. A detailed analysis of the location of free thiols demonstrated a remarkable selectivity of free thiol formation occurring in the CH2 domain. Glycosylation of the Fc CH2 domain is a conserved feature of IgG antibodies expressed in mammalian cells, whereas it is absent in E. coli-expressed antibodies. Therefore, we hypothesized that lack of Fc glycosylation might be responsible for the observed susceptibility to selective free thiol formation in the CH2 domain.

To evaluate this hypothesis, we first established a small lab-scale model of free thiol induction using an E. coli cell homogenate. Subsequently, IgG1 antibodies expressed in CHO cells were tested for their susceptibility to free thiol formation after complete deglycosylation with PNGase F or after partial glycan trimming with glycosidases. Analysis of the total free thiol content demonstrated that glycosylated antibodies were largely resistant to free thiol formation using the free thiol induction model. Complete removal of Fc glycans by PNGase F resulted in a significant increase of the total free thiol content. Partial trimming of the Fc glycans with exo- and endoglycosidases resulted in variable susceptibility to free thiol formation, depending on the size of the glycans present, with smaller glycans having a smaller protective effect. Finally, the location of the free thiols was determined using a differential isotope tagging strategy in conjunction with LC-MS peptide mapping, which confirmed that the free thiols were predominantly induced in the CH2 domain of the tested antibodies.

These results strongly indicate that the selective free thiol formation observed in the CH2 domain of an E. coli-expressed IgG1 antibody was the result of the lack of Fc glycosylation. They also suggest a potential new protective role for Fc glycans in IgG1 antibodies.

(395) Maltohexaose based contrast agents detect bacterial infections

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Bacterial infections are a central cause of mortality in the world and affect all areas of medicine ranging from cardiology to oncology. Bacterial infections remain a major health problem despite the availability of effective antibiotics, not only because their diagnosis is challenging and but also because they are frequently treated with ineffective antibiotics, due to the widespread rise of bacterial drug resistance. In this report, we present a new PET contrast agent, composed of F-18 conjugated to maltodextrins (^{18}F-MD), which can for the first time image bacteria in vivo with the specificity and sensitivity needed to detect early stage infections and measure drug resistance in vivo (see Figure 1 for structure). We show here that ^{18}F-MD can detect as few as 105 E.coli colony forming units (CFUs) in rats, which is 3-4 orders magnitude higher in sensitivity than ^{18}F-FDG, a clinically used PET tracer. In addition, we demonstrate that 18F-MD can distinguish bacterial infections from inflammation, and has a specificity that is 2-3 orders of magnitude higher than ^{18}F-FDG, giving it the potential to identify infections clinically without a biopsy. Finally, we demonstrate that ^{18}F-MD can monitor treatment efficacy *in vivo* and can identify beta lactam resistance in *E.coli*, thus providing physicians with a powerful tool for guiding antibiotic selection. We anticipate numerous clinical applications of ^{18}F-MD given the widespread use of PET and the pervasiveness of infections in medicine.

(396) Properties of a rare sugar D-allulose (D-psicose)

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Rare sugars were defined as monosaccharides and their derivatives those are rare in nature by the International Society of Rare Sugars (ISRS) in 2002. One of the rare sugars D-allulose (also called D-psicose) is an epimer of D-fructose, and is as sweet as D-glucose. This rare sugar has been evaluated as a unique metabolic regulator of glucose and lipid metabolism, and thus represents a promising compound for the treatment of type 2 diabetes mellitus (T2DM). In this work, we examine the properties of D-allulose both in vivo and in vitro.

T2DM model rats were used for the evaluation of the efficacy of D-allulose on this disease. Rats were fed 5% D-allulose supplemented in drinking water for 13 weeks. The reduced increase in body weight, abdominal fat mass, blood glucose and insulin level were observed. Histochemical staining of liver and pancreas showed that D-allulose prevented the progress of T2DM and obesity.

Basic kinetic study was performed with healthy rodents. Using 14C-labelled D-allulose and Wister rats, its absorption, distribution and elimination were studied. The concentrations in whole blood, urine and each organ were measured. Half of the intravenously administered D-allulose (100mg/kg) was transferred from blood to urine within 120 min. The half-life of D-allulose from the blood was 57.3 min. Accumulation in organs was not observed except for in the liver. With a single dose oral administration (100mg/kg), D-allulose was easily absorbed from the gastrointestinal tract to blood. Trace amount of radioactivity was detected in the liver and small intestine 7 days after oral administration.

Autoradiography was also performed using C3H mice. Similar to the results obtained with rats, high signals of 14C-labelled D-allulose were observed in liver, kidney and ladder. Interestingly, no accumulation was observed in the brain.

In vitro study with human serum albumin revealed that D-allulose adsorbed immediately with the molar ratio of 1:1. And the absorbed amount did not increase for 4 weeks. The absorbed amount was less than D-glucose.

In conclusion, D-allulose might be useful to prevent hyperglycemia and /or obesity in pre-diabetic patients.

(397) Bis(β-lactosyl)-fullerene as novel class of glycolipids useful for detection and decontamination of biological toxins in Ricinus communis family

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Carbohydrate recognition proteotoxins, such as verotoxins and cholera toxins, often cause serious damages to human cells. Ricin, a proteotoxin isolated from the castor bean (*Ricinus communis*), is one of the strongest toxins and has been employed for bioterrorism. In the recent quarter of a century, the development of proteotoxin infection inhibitors using carbohydrate ligands has attracted much interest. In particular, multivalent biomaterials composed of molecules containing two or more carbohydrate ligands have been designed to enhance protein-carbohydrate interactions through glycocluster effects. To further contribute to these research efforts, glycosyl fullerenes that mimic glycolipids have been developed. In aqueous media, these fullerene derivatives form liposome-like aggregates that show a stronger affinity to carbohydrate recognition proteins, such as concanavalin A (Con A), compared to corresponding monomeric sugar unit. In this study, bis(β-lactosyl)-[60]fullerene (bis-Lac-C_{60}) was designed and synthesized for detection and decontamination of ricin.

A fullerene derivative having two β-lactose units was synthesized via [3+2] cycloaddition between C_{60} and the terminal azide group of the lactose derivative. Bis-Lac-C_{60} solution was obtained by deacylation of the fullerene derivative and subsequent dialysis of the DMSO solution against distilled water. Bis-Lac-C_{60} displayed specific and strong binding affinity to ricin and its family RCA120, which consequently led to form insoluble aggregation immediately. In contrast, no sedimentation was observed when Con A or PBS buffer was added to the bis-Lac-C_{60} suspension. The *in vitro* decontamination ratio of aqueous ricin solutions was estimated its distribution in each supernatant and aggregate after the sedimentation. The ratio has reached 94% when 100 QM bis-Lac-C_{60} and 150 Qg mL-1 ricin solutions were mixed, showing that bis-Lac-C_{60} acted as a highly efficient decontaminant against ricin. Brine addition to the ricin/bis-Lac-C_{60} suspension accelerated the sedimentation and enhanced the in vitro decontamination efficiency. The optimized efficiency against 150 mg mL-1 ricin solution surpassed 99% upon treatment with 363 QM bis-Lac-C_{60} and 50 mM brine solutions.

(398) Rare sugar D-allulose (D-psicose) prevents progression and development of diabetes in Type 2 Diabetes Mellitus (T2DM) rat model

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Overweight and obesity have emerged as the leading risks for global deaths. Excess calorie intake initiates this tragedy with the development of insulin resistance, followed by concomitant increase of T2DM. This circumstance demands age-adjusted balanced food intake in obese-tendency subjects. We introduce a zero-calorie sweet sugar, D-allulose, a rare sugar produced in Kagawa University, Japan, which has been evaluated having strong anti-hyperglycemic and anti-hyperlipidemic effects and thus represents as a safe and non-toxic compound to maintain blood glucose levels and to control body fat. 5% D-allulose supplemented in drinking water was fed to treated T2DM model Otsuka Long-Evans Tokushima Fatty (OLETF) rats, and only water to control OLETF and non-diabetic Long-Evans Tokushima Otsuka (LETO) rats for 60 weeks. Body weight, food intake, blood glucose and serum insulin levels were measured periodically. Oral glucose tolerance test (OGTT) was performed in several intervals. Liver, pancreas and other organs were preserved and stained as per need. D-allulose prevented the progression and development of diabetes through constant maintenance of blood sugar levels. OGTT showed reduced blood glucose levels suggesting the improvement of insulin resistance. D-allulose controlled abdominal fat accumulation and thus prevented excess body weight increase. D-allulose also attenuated pancreas islet fibrosis with the preservation of islets, evaluated by several staining methods and immunostaining of insulin and a-smooth muscle actin. Rare sugar D-allulose might be a promising compound for the prevention of obesity and diabetes.

(399) Large Scale Production of Aggrecan from Salmon Nasal Cartilage and Medical Applications –Special Reference to Aggrecan Micro-needle-

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We succeeded in the large scale production of highly purified aggrecan from salmon nasal cartilage. The conditions of extraction and purification was precisely examined and the procedure was established with high yield. The chondroitin sulfate residues and protein part of the purified aggrecan were characterized. The purified aggrecan was examined for confirmation of the biological functions reported previously. The aggrecan was found to form very smooth thin film. Taking the advantage of this physical property, we tried to prepare a micro-needle with the aggrecan. The prepared micro-needle contains 144 needles with 800 Qm length within 1cm2. The surfaces of the needles are quite smooth and sharp. It is one of the major advantages of the needle because the needles are easily transferred to the skin tissue without pain. The needle parts were found to be dissolved in the skin tissue within 2hours and the chemicals embedded in the needle parts were effectively dispersed to the neighboring epidermis and dermis. On the basis of this property, we tried to produce antibody against embedded antigen, ovalbumin in the micro-needle. The obtained antibody titers were higher than those by normal injection of the antigen with stainless needle. Furthermore, with the combination of IFN-Ð and the antigen, the obtained antibody titers increased very much and comparable with those obtained by the immunization with the addition of Montanide adjuvant. We found many advantages of this micro-needle and possible medical applications will be presented.

(400) A New Functional Sweetener D-Psicose (D-Allulose) With Zero Calorie Has High Potential To Change Our Life Style

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"Rare sugars" are monosaccharides (minimal functional unit of sugars) that are rarely found in nature, but existing about 50 types. Kagawa University has been successful in producing all of the rare sugars, and has the only research group in the world that prioritized research and development of rare sugars, which has resulted in the ability to produce a high quantity and quality of these precious "Rare sugars".

D-Psicose (D-Allulose), one of rare sugars, is a palatable and refreshing sweetener with zero calorie, whose functions include reducing blood sugar levels and lowering lipid accumulation to the body. The former function may be useful for prevention and/or treatment of pre-diabetic and diabetic patients, and the latter for that of atherosclerosis and obesity. It is now under review for the government approval as a functional food for specified health use (FOSHU), while there are many food products containing D-Psicose sold on the market such as cakes, bread, and other sweets, soup and beverages in Japan. Japan is the only nation that has legally defined functional foods for health, and the Japanese functional food market is one of the most advanced in the world. For those reasons, developments in Japan are often cited as indication of possible developments for Asia, Europe, the United States and the rest of the world.

Another rare sugar, D-Allose shows another functional benefit to reduce the production of oxygen radicals. The beneficial effect has been shown, for example, to prevent the onset of salt-induced hypertension in rat models. As there are many diseases such as metabolic diseases, cardiovascular diseases, neurodegenerative diseases where oxygen radicals are involved in their pathogenesis, D-allose may be widely used for applicative purposes as a functional food or medicine.

Rare sugars are new alternative sweeteners. Utilizing rare sugars effectively in our daily life will be crucial in establishing a "Healthy and Sustainable Society".

(401) Increased N-glycosylation of Asn88 in serum pancreatic ribonuclease 1 is a novel diagnostic marker for pancreatic cancer

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Alterations of carbohydrate structures in cancer cells are the most promising targets for developing clinical diagnostic reagents. In the conventional glycan markers for tumor such as core-fucosylation of alpha-fetoprotein, sialyl lewis A and X antigens, the changes in peripheral structures of glycan chains were mainly focused. In contrast to global structural changes, there is no reported study of the presence or absence of N-glycan linked to a specific Asn residue within glycoproteins that correlate with diseases, with the exception of genetic disorders of glycosylation.

In this study, I report the increase of N-glycosylation at a specific asparagines residue, Asn^{88}, of the serum pancreatic ribonuclease 1 (RNase 1) in patients with pancreatic cancer, and the development of a serum cancer marker for detecting pancreatic cancer. Two antibodies were raised against human RNase 1 that bind to the enzyme containing unglycosylated Asn88, but not when its Asn^{88} is N-glycosylated. The immunoassay of ELISA and Western blot analyses showed a significant increase in the serum levels of RNase 1 containing N-glycosylated Asn^{88} in pancreatic cancer patients compared with normal human subjects. Furthermore, two enzyme immunoassay reagents were developed for the accurate measurement of the levels of total RNase 1 and one with unglycosylated at Asn^{88} (Asn^{88}-free RNase 1) in serum. The "G3/t ratio" calculated from the levels of total and Asn88-free RNase 1 showed good performance for detecting pancreatic cancer (area under curve value: 0.795, specificity: 75.8% and sensitivity: 91.7%). Focusing on the increase in an N-glycosylated Asn residue of serum RNase 1, specifically Asn88, affords a new diagnostic marker for pancreatic cancer, one of the most difficult cancers to diagnose because it lacks definitive symptoms.

This is the first diagnostic cancer marker that takes advantage of the presence or absence of N-glycosylation at a specific Asn residue of a glycoprotein.

(402) poFUT1 promotes trophoblast cell proliferation through activating MAPK and PI3K/Akt signaling pathways

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Protein O-fucosylation is one of the important types of glycosylation which play important roles in embryonic development. Protein O-fucosyltransferase 1 (poFUT1) is an essential enzyme that catalyzes the synthesis of protein O-fucosylation. Our previous studies have showed that poFUT1 promotes trophoblast cell migration and invasion at the fecal-maternal interface, but the role of poFUT1 on trophoblast cell proliferation are still not clear. The present results showed that poFUT1 promote trophoblast cell proliferation by CCK-8 assay and cell cycle analysis. PoFUT1 increases the phosphorylation of ERK1/2, p38 MAPK, and PI3K/Akt. Inhibitors of ERK1/2(PD98059), p38 MAPK(SB203580), and PI3K (LY294002) prevented the phosphorylation of ERK1/2, p38 MAPK, and Akt. In addition, poFUT1 stimulate trophoblast cell proliferation correlates with increased cell cycle progression by promoting cells into S-phase. The mechanism involves in increased expression of cyclin D1, cyclin E, CDK 2, CDK 4, and pRb, and decreased level of cyclin-dependent kinases inhibitors p21 and p27, which are blocked by the inhibitors of upstream signal molecules, MAPK and PI3K/Akt. In conclusion, poFUT1 promotes trophoblast cell proliferation through activating MAPK and PI3K/Akt signaling pathways. [Acknowledgments: This work is supported by National Natural Science Foundation of China Research Grant (31270866, 31200606), and Specialized Research Fund for the Doctoral Program of Higher Education of China (20122105120003), and National Natural Science Foundation of Liaoning (2014023045).And Program for Liaoning Excellent Talents in University (LNET).]

(403) The metabolism of glycosphingolipids and sphingolipids in Farber disease

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Acid ceramidase (AC) is the a lysosomal hydrolase encoded by the ASAH1 gene, which catalyses the hydrolysis of ceramide to sphingoid base and fatty acid. Deficiency of AC activity leads to the lysosomal storage disorder known as Farber disease (FD). The main clinical features are painful and progressively deformed joints, subcutaneous nodules and premature death. The clinical diagnosis of FD is usually confirmed by biochemical methods, such as the determination of ceramide accumulation and/or measurement of the AC activity. The pathogenesis mechanism of FD is still unclear. Recent studies showed that AC appears to modulate cell functions by controlling the levels of ceramide and sphingoid bases, which are both considered as putative bioactive molecules. To clarify the pathogenesis of FD, we focused on the contents of the molecular species of glycosphingolipids and sphingolipids. We compared the contents of the carbohydrate moiety of glycosphingolipids and the sphingolipids class including molecular species between the fibroblasts from FD patient (FD cells) and healthy subject (NB cells). We also created the human AC expression vector and established AC overexpressing FD cells, which have the same level of AC activity compared with NB cells. Lipidmics analysis demonstrated that the contents of ceramide and hexosylceramide were markedly increased and that of sphingomyelin and sphingosine-1-phosphate were significantly decreased in FD cells, and these changes were recovered in AC overexpressing FD cells. The specific changes of molecular species were not observed in each class of sphingolipid.

(404) Glycoprofiling of breast cancer and changes in glycan expression throughout progression of tumour states

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Introduction: Breast cancer is the leading cause of cancer deaths among women in developed countries. In some patients, the cancer is cured by surgical elimination of the primary tumor, however, other patients develop recurrences, either locally or in distant regions of the body.

Aberrant glycosylation is known to be present in the progression of many types of cancers and changes in glycosylation correlate with tumour growth, adhesion and metastasis. Protein glycosylation is predominant in membrane proteins, moreover, changes in glycosylation of these proteins have been shown to correlate with cancer states. Thus, identifying changes in glycan expression throughout progression of cancer could provide more specific and sensitive cancer biomarkers.

Methods: Triton-X114 phase partitioning was used for extraction of membrane proteins after which the N-glycans were released and analyzed by PGC-LC-ESI-MS/MS.

Results: Cell wide N-glycan profiling of breast cancer cells was carried out. Glyco-profiles were compared on proteins from normal-like and luminal cell lines grown in cell culture and are now being compared to the corresponding cells derived from mice xenografts.

Conclusion: Glycosylation changes on membrane proteins of breast cancer cells vary depending on cell origin.

(405) Alteration or adaptation, the two roads for human gastric mucin glycosylation infected by *Helicobacter pylori*

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Helicobacter pylori is a gram negative spiral bacterium which colonizes human stomach and infects more than half of the world's population. Although most people are asymptomatic, persistent infection may cause different affections like gastritis, duodenal ulcer, intestinal metaplasia or gastric cancer.

Adhesion of the bacteria to the gastric mucosa is a necessary prerequisite for the pathogenesis of *H. pylori*-related diseases and is mediated by bacterial adhesins, which recognize blood group antigens and other motifs carried by O-glycans of the host. Although BabA and SabA are the most prevalent adhesins studied so far, not all *H. pylori* strains express these adhesins functionally. This implies that other bacterial entities must be involved in adhesion of the bacteria to gastric mucin O-glycans.

By proteomic analysis and mutant construction, we have characterized a new adhesin of *H. pylori*, termed LabA, that binds specifically the lacdiNAc motif located on MUC5AC mucins. Localization of the LabA target, restricted to the gastric mucosa, suggests a plausible explanation for the tissue tropism of these bacteria.

To better understand the physiopathology of the infection, we further purified human gastric mucin O-glycans and used them in inhibitory adhesion assays to identify new targets of *H. pylori* in the gastric mucosa. To determine whether these new *H. pylori* binding glycans were differently expressed on mucins during the course of infection, mucins were purified from gastric tissues of 25 infected asymptomatic individuals, 10 healthy uninfected individuals and 5 infected patients with incomplete type of intestinal metaplasia in the gastric mucosa. The glycosylation of gastric mucosa of asymptomatic individuals infected by *H. pylori* was determined and compared with the glycosylation pattern found for patients with incomplete type of intestinal metaplasia. Results show that *H. pylori* manages to modulate host's glycosylation during the course of infection in order to create a favorable niche whereas asymptomatic infected individuals seem to counteract further steps of infection development by adapting their mucus glycosylation. These results pave the way for the development of alternative strategies against *H. pylori* infection, using adherence inhibitors.

(406) Identification of mucin glycans recognized by microorganisms: development of a new adhesion assay

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Mucus composed of highly hydrated mucins is secreted by specialized epithelial cells in the light of the gastrointestinal tract, lungs, urinary and reproductive systems, constituting a protective barrier against bacterial infections. Mucins are long linear proteins carrying hundreds of oligosaccharide chains that harbor a high diversity of glycan motives containing GalNAc, GlcNAc, Gal, Fuc and sialic acids combined according to various linkages giving rise to a plethora of structures. Microorganisms like *Helicobacter pylori, Pseudomonas aeruginosa,* pathogenic *Escherichia coli* and potentially *Aspergillus fumigatus* developed a capacity to bind mucins *via* their glycan motives to facilitate host colonization.

We developed a binding test so to study the adhesion capacities of bacteria to mucins and performed competitive inhibition tests aiming at determining the influence of the different glycan chains and motives on bacterial binding. Purified mucins were blotted on a nitrocellulose membrane before incubation with DAPI labeled bacteria and fluorescence detection.

H. Pylori show a strain specific binding profile to mucins. Some clinical strains bind preferentially to mucins from blood group Lewis a patients whereas others rather bind to mucins of Lewis b individuals.

The notion of adherent/non-adherent *E. coli* strains was reassessed. Indeed, even though *E. coli* is part of the intestine commensal flora, some strains are highly pathogenic and can be lethal. These strains possess glycan specific adhesins playing a crucial role in their pathogenicity. Up to now, *E. coli* strains have been classified into adherent or non-adherent *E. coli* according to the exclusive binding of bacteria at the cellular surface, excluding mucins from the criteria. Using mucins from several human intestines, we show that even strains considered to be non-adherent actually strongly bind to human intestinal mucins. Binding capacities are influenced by the glycan motives harbored by the mucins.

Concerning *Pseudomonas aeruginosa*, the pathogen showed a predilection for sialylated lung mucins rather than for neutral mucins. When bacteria were preincubated with sialic acid, binding to mucins was significantly reduced, suggesting that sialic acid is part of the motif recognized by the *P. aeruginosa*. In the opposite, the opportunistic fungi *Aspergillus fumigatus* preferentially binds to neutral mucins.

(407) A Glycogene mutation map (GlyMAP) for discovery of diseases of glycosylation

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The number of disorders collectively classified as Congenital Disorders of Glycosylation (CDG) has increased during the recent years. Classical CDG diagnostics is conducted via various glycan profiling techniques and the predominant forms of the reported CDG's are severe multisystemic disorders. Thus, most known CDGs are caused by defects in glycogenes that effect glycosylation globally. Glycosylation of proteins and lipids involves over 200 known glycosyltransferases, and deleterious defects in many of these genes underlie many of the CDGs reported. Many glycosyltransferases are members of homologous isoenzyme families and deficiencies in individual isoenzymes may not affect glycosylation globally. In line with this there appears to be an underrepresentation of disease-causing glycogenes among these larger isoenzyme homologous families. However, Genome-Wide-Association Studies (GWAS) have identified such isoenzyme genes as candidates for different diseases, but validation is not straightforward without biomarkers. Large-scale whole exome sequencing (WES) provides access to mutations in e.g. glycosyltransferase genes in populations, which can be used to predict and/or analyze functional deleterious mutations. Here, we constructed a draft of a Functional Mutational Map of glycogenes, GlyMAP, from WES of a homogenous population of 2,000 Danes. We catalogued all missense mutations in 208 glycosyl transferase genes and used prediction algorithms, manual inspection, and in case of CAZy family GT27 experimental analysis of mutations to map deleterious mutations. Our approach has revealed that deleterious glycogenes alleles do exist in the general population studied. GlyMAP[1] provides a first global view of the genetic stability of the glycogenome and could serve as a tool for discovery of novel CDGs. Current efforts are now aimed at extending our approach to include all genes involved in shaping the human glycome and as a first step in this direction the gene encoded hydrolases will be included in our analysis.

[1]"A Glycogene Mutation Map (GlyMAP) for Discovery of Diseases of Glycosylation". Lars Hansen, Allan Lind-Thomsen, Hiren J. Joshi, Nis Borbye Pedersen, Christian Theil Have, Yun Kong, Shengjun Wang, Thomas H. Sparsøe, Niels Grarup, Shengjun Wang, Malene Bech Vester-Christensen, Katrine Schjoldager, Torben Hansen, Oluf Borbye Pedersen, Bernard Henrissat, Henrik Clausen, Hans H.Wandall and Eric P. Bennett. Re-submitted to Glycobiology for re-revision, Sept. 19, 2014.

(408) Docking and design of oligosaccharides, glycoproteins, and glycolipids: Expanding the computational tools available to glycoscientists

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The challenge of modeling carbohydrates is infamous, due to inherent conformational freedom. Glycans affect protein structure, stability, and activity, but the molecular mechanisms are typically unpredictable. We seek to develop fast, accurate, and high-throughput methods of both modeling and designing carbohydrates for applications in glycobiology. We have built a framework within Rosetta, a structural modeling and design suite, for modeling saccharide ligands and glycosylated proteins and lipids. Our efficient and intuitive data structures capture the high degree of flexibility, stereochemistry, and branching in carbohydrates and allow access to main-chain and side-chain torsions (φ, ψ, ω, and χ) and Cremer-Pople parameters for sampling ring forms. Rosetta's speed and flexibility enable modeling of any glycan-containing molecule in docking and refinement protocols through exploring this vast torsional and ring-conformational diversity.

The residue-centric approach of Rosetta, combined with combinatorial "patching" of standard residues with specific chemical moieties, allows for design algorithms in which alternative sugar units are sampled, enabling high-throughput screening of thousands of glycoforms and protein variants in a search for stable or functional molecules.

Here, we will present three studies benchmarking monosaccharide ring conformations, oligosaccharide structure prediction, and bound-bound protein-oligosaccharide docking. We examine the relative energy surfaces for all D-aldohexopyranose ring forms; we evaluate predicted structures of Lewis^{X} and SiLewis^{X}; and we compare the docking predictions of eleven glycoantigen-antibody pairs with known structures. These studies will allow us to rigorously test the Rosetta scoring (energy) function, adapting it for the unique chemical and electronic effects of carbohydrates.

We will also present preliminary real-world applications in the activity of glycosylated carboxylesterases, antibody accessibility for glycosylation enzymes, and x-ray crystal refinement of an extensively glycosylated HIV-1 envelope protein trimer.

These new approaches will provide glycobiologists and glycoengineers a new computational toolbox that will further the understanding of the biomolecular mechanisms of disease and create opportunities for a wide range of previously intractable studies.

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