The 8th Symposium
of Japanese Consortium for Glycobiology and Glycotechnology

Novel approaches for glycan functions and their medical applications

November 29 - 30, 2010 at Tokyo Conference Center (Shinagawa)

Program November 29 (Monday), 2010

Opening Address
Toshisuke Kawasaki (JCGG President, Ritsumeikan University)

Greetings from Organizer
Akemi Suzuki (Tokai University)

Session 1 Development of Technology for Glycan Synthesis and Its Application - (1)
Chair : Akemi Suzuki (Tokai University)

Synthetic bioorganic study on artificial glycoconjugates
Toshiuki Inazu (Tokai University)

We have been studying the synthesis of artificial glycoconjugates and the elucidation of their functions.

As an example of this line of investigation, we synthesized the artificial insulin with an N-linked oligosaccharide by a chemo-enzymatic method using endo-N-acetylglucosaminidase (ENGase) (EC 3.2.1.96) from *Mucor hiemalis* (Endo-M). GlcNAc-modified insulin was prepared by the reaction of the carboxymethyl glycoside of GlcNAc and bovine insulin using a dimethylphosphinothioic mixed anhydride (Mpt-MA) method. A transglycosylation reaction of the GlcNAc-modified insulin using Endo-M gave mono-transglycosylated insulin predominantly. We determined the transglycosylation site of the mono-transglycosylated insulin by MALDI-TOF MS of the peptidase digested fragments.

As another study, we determined the structural specificity of the glycosyl acceptor of the transglycosylation reaction of several synthetic acceptor derivatives using Endo-M. The narrow regions of the 1,3-diol structure from the 4- to 6-hydroxy functions of GlcNAc were found to be essential for the transglycosylation reaction using Endo-M. Furthermore, it was determined that Endo-M strictly recognizes a 1,3-diol structure consisting of primary and secondary hydroxyl groups.

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Progress in the field of peptide science for glycoprotein synthesis

Hironobu Hojo (Tokai University)

In this presentation, we present our recent result of (glyco)protein syntheses using ligation methods. One is an efficient synthesis of hydrophobic protein, saposin C, by the native chemical ligation method combined with the O-acyl isopeptide method, which is known to increase the solubility of peptides. The N-terminal peptide thioester having O-acyl isopeptide structures was prepared by the Boc mode solid-phase peptide synthesis (SPPS) using azido protection at the isopeptide site. Then the segment was ligated with C-terminal peptide with an *in situ* reduction of the azido group followed by an O- to N-acyl shift to successfully obtain saposin C. The other is the glycoprotein synthesis by the thioester method. This method has the advantage that any residue can be selected as a ligation site. Instead, the side chain amino and thiol groups have to be protected. To realize this, the Boc groups have to be reintroduced to the side chain amino groups, which were made free during the deprotection after SPPS. We examined the direct synthesis of the side chain-N- protected peptides by introducing azido-protected Fmoc-Lys during SPPS to overcome the inconvenience. This improved method was successfully applied to the synthesis of N- and O-glycosylated pro-opiomelanocortin (1-74).

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Nobel approach to glycoprotein analysis assisted by chemical modifications

Akihiko Kameyama (AIST)

Mass spectrometry (MS) has become a powerful technique for the characterization of glycans and glycoproteins. However, characterization and detection of sulfated glycopeptides by MS is difficult due to the low abundance and low ionization efficiency of these molecules. To overcome the problems, we developed a novel enrichment procedure for sulfated glycopeptides. The procedure consists of anion

exchange chromatography and a sulfate emerging (SE) method which controls the net charge of peptides by utilizing limited proteolysis and modification with acetohydrazide. Using this procedure, we discovered three sulfated glycoproteins from a culture medium of a lung cancer cell line.

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Facile method for synthesis of 2-acetamido-glycosies via chemo-enzymatic process

Shin-ichiro Shoda (Tohoku University)

Unprotected glycosyl compounds having a leaving group at the anomeric position are useful glycosyl donors for chemo-enzymatic glycosylation. Here we report the direct synthesis of 4,6-dimethoxy-1,3,5-triazin-2-yl glycoside and sugar oxazolines starting from the corresponding 2-acetamido-sugars. These compounds were found to be efficient glycosyl donors for glycosidase-catalyzed transglycosylations.

An activated N-acetylglucosamine having an anomeric 4,6-dimethoxy-1,3,5-triazine moiety with an α-configuration has been prepared in good yield in water without using any protecting groups. When α-N-acetylglucosaminidase was incubated with 4,6-dimethoxy-1,3,5-triazine-2-yl α-N-acetylglucosaminide (GlcNAc-α-DMT) as glycosyl donor and *p*-methoxylphenyl β-galactoside as glycosyl acceptor, the transglycosylation products were obtained in fairly good yields. α-Linked N-acetylglucosamine is primarily found in heparin sulfate and gastric gland mucous cell-type mucin. Recently, α-GlcNAc-capped O-glycans in the mucin have received much attention as an antimicrobial agent against *Helicobacter pylori*.

We have developed a new synthetic method that can directly convert reducing GlcNAc of various glycans to the corresponding oxazoline moiety without the protection and deprotection by using 2-chloro-imidazolinium chloride as dehydrative agent. A simple and efficient chemo-enzymatic synthesis of various sialo-complex type glycoformed bioactive peptides and proteins has successfully been achieved by the combined use of Endo- M-Nl75Q mutant and 'one-pot' synthesized sialocomplex type sugar oxazoline.

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JCGG Luncheon Seminar 1 (sponsored by Shimadzu Co.)

Functions of sulfated glycans for the maintenance and the differentiation of ES cells and iPS cells

Shoko Nishihara (Soka University)

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Session 2 Functional Analyses Involved in Glyan Related Molecules

Chair : Toshiyuki Inazu (Tokai University)

Development of novel oligomannose-coated liposome-based vaccines

Naoya Kojima (Tokai University)

Strategies that target APCs and modulate APC function *in vivo* have significant implications for vaccine design. APCs express many C-type lectin receptors (CLRs) that act as phagocytic receptors. In addition, some CLRs trigger distinct signaling pathways that induce expression of specific cytokines. Thus, targeting of CLRs on APCs is a promising way to deliver antigens to APCs and to control APC function, and carbohydrate-decorated particles such as carbohydrate-coated liposomes with encapsulated antigens might be a suitable antigen delivery vehicle to target CLRs on APCs. We introduce oligomannose-coated liposomes (OMLs) as novel antigen-delivery vehicles that also have a strong adjuvant effect on induction of Th1 immune responses and CTLs specific for the encased antigen. This property of OMLs is due to specific cellular uptake and an ability to promote APC maturation, presentation of antigens on MHC class I and class II molecules, secretion of IL-12 from APCs, and migration of APCs into lymphoid tissues from peripheral tissues. Feasibility studies

of OML-based vaccines using mouse models have revealed their potential for clinical use in vaccination for diseases in which CTLs and/or Th1 cells act as effector cells.

Structural studies of intracellular lectin OS-9 involved in ER quality control

Tadashi Sato (RIKEN)

N-Glycans play an important role as a destination signal in the endoplasmic reticulum (ER) quality control, and intracellular lectins are known to regulate these process. Correctly folded glycoproteins can be transported to Golgi apparatus by transport lectins such as VIP36. On the other hand, misfolded glycoproteins are translocated from the ER into the cytosol for proteasome-mediated degradation. A mannose-6-phosphate receptor homology (MRH) domain is commonly identified in a variety of proteins, and, in the case of OS-9, is involved in glycoprotein ER-associated degradation (ERAD). Trimming of outermost al,2-linked mannose on C-arm of high mannose-type glycan and binding of the trimmed α1,6-linked mannosyl residues by the MRH domain are critical steps in guiding misfolded glycoproteins to enter ERAD.

Here we report the crystal structure of a human OS-9 MRH domain complexed with α3,α6-mannopentaose, which is a minimal mannosyl residues of glycoprotein ERAD substrates. The OS-9 MRH domain has a flattened β-barrel structure with a characteristic P-type lectin fold, and possesses distinctive double tryptophan residues in the sugar-binding site. Our crystallographic result in conjunction with NMR spectroscopic and biochemical results provide structural insights into the mechanism whereby OS-9 specifically recognizes Manα1,6Manα1,6Man residues on the Manα1,2-trimmed C-arm through the WW motif.

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Accumulation of lactosylceramide and neuronal cell death
Junko Matsuda (Tokai University)

Lactosylceramide (LacCer) is a pivotal intermediate in synthesis and lysosomal degradation of complex glycosphingolipids (GSLs). Two lysosomal β-galactosidases, GalCer-β-galactosidase (Gale) and GM1-β-galactosidase (Bgal), and sphingolipid activator proteins (saposins A, B, and C) participate in its degradation. LacCer has been found to be increased in various tissues of several lysosomal storage disorders (LSDs). To understand the pathophysiological roles of LacCer in LSDs, we generated Twitcher mouse (*Galc*$^{-/-}$), which is an authentic mouse model of human Krabbe disease with additional deficiency of saposin C (*Galc*$^{-/-}$*Sap*-C$^{-/-}$) by crossbreeding experiment. Ga1-c-1 sap -c- showed much shorter life span than that of Gal-e- with the massive accumulation of LacCer in the brain. Neuropathologically, in addition to the demyelination, *Galc*$^{-/-}$*Sap*-C$^{-/-}$ exhibited a rapidly progressive neuronal cell degeneration which have rarely seen in *Galc*$^{-/-}$. Neuronal cell deaths were characteristically observed in the hippocampal CA2, entorhinal cortex, caudate/putamen, olfactory tubercle, thalamus, and cerebellar granular layer, along with the reactive astrogliosis. Immunohistochemically, antibody against LacCer revealed the accumulation of LacCer in the neurons and astrocytes in the above described regions. We found the temporal and spatial correlation between LacCer accumulation and neuronal cell death in *Galc*$^{-/-}$*Sap*-C$^{-/-}$, indicating the neurotoxicity of LacCer.

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Glycans and glycolipids supporting functions of membrane molecules
Akemi Suzuki (Tokai University)

The structure and function of glycosphingolipids (GSLs) and membrane glycoproteins are our research subject and we focus on the structure of GSLs of microvillous membrane of pig small intestine and the modulation of ligand binding activity of megalin by N-glycans. The mucosa of pig small intestine contained 5 major neutral GSLs, B1 to BS. B3 and B4 were identified to be Gb3 and Gb4, both containing sphingenine and normal fatty acid (nFA) and B5 to be Fuc-nLc4 containing 4-hydroxysphinganine and hydroxy FA. Mouse kidney expresses megalin, 600kDa glycoprotein, in proximal tubular epithelial cells. Fractionated megalin by LCA and WGA agarose from a detergent-solubilized membrane of mouse kidney demonstrated that LCA-bound megalin shows higher ligand binding activity than WGA-bound megalin, indicating that N-glycan can modulate ligand binding activity of megalin. LC-IT-MS (liquid chromatography-ion trap-mass spectrometry) was

applied to ganglioside analysis of mouse spleen and brain, demonstrating that it will be a powerful method to analyze ganglioside structure and content in isolated cells, microdomains, and small amount of tissues.

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Functional and structural analysis of glycolipid enriched microdomain

Kazuya Kabayama (Tokai University)

Membrane lateral heterogeneity is accepted as a requirement for the function of biological membranes, and the notion of "raft/microdomain" gives specificity to this concept. Recently, fluorescence-based techniques such as fluorescence recovery after photobleaching (FRAP), single particle tracking (SPT) and fluorescence correlation spectroscopy (FCS) were promising for application to the dynamics of membrane molecules in microdomain. We hope to demonstrate that an alteration of lipid component in microdomain affects lateral diffusion of membrane receptor. Therefore, we established experimental system in which monitored the membrane organization of receptors by analyzing its lateral diffusion parameters in the plasma membrane of living cells using FRAP, SPT. In this study, measurement of the lateral diffusion of IR was performed by fitting analysis to fluorescence recovery curves and trace analysis to individual fluorescent spots, which provided diffusion coefficient (D). It shows us how fast membrane molecules are diffusing at the change of membrane environment such as before and after stimulation by cholesterol depression. We will utilize these techniques for the lateral diffusion analysis of membrane receptor in another assay conditions, such as treatment of glycosphingolipid (GSL) inhibitor, use of GSL deficient cells or of pathologic samples.

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Session 3　New Findings of Glycan Functions (1)

Chair : Junko Matsuda (Tokai University)

Molecular function of testis specific glycolipid, seminolipid

Koichi Honke (Kochi University)

A sulfoglycolipid, seminolipid is specifically expressed in mammalian spermatogenic cells. Its sulfation is catalyzed by a glycan sulfotransferase, cerebroside sulfotransferase (CST). Disruption of the CST gene in mice results in the complete absence of seminolipid in the testis and male infertility due to the arrest of spermatogenesis at the late stage of the prophase of the first meiosis, indicating that seminolipid is essential for spermatogenesis (1). Basigin (EMMPRIN/CD147) is an immunoglobulin superfamily protein and expressed in spermatogenic cells. Basigin-deficient male mice are also azoospermic, and their germ cells are mostly arrested at the metaphase of the first meiosis and degenerated (2). Since the phenotype in spermatogenesis is similar between seminolipid-KO and basigin-KO mice, we investigated interaction between seminolipid and basigin in germ cells to elucidate their roles in spermatogenesis. Immunohistochemical analysis, DRM fractionation and immunoprecipitation experiment suggest that seminolipid and basigin function cooperatively and play a key role in spermatogenesis.

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Palmitoylated Ras proteins localize at recycling endosomes

Ryo Misaki (Osaka University)

Ras proteins are small GTPases that regulate cell growth, death, and differentiation. The two ubiquitously expressed Ras isoforms, H- and N-Ras are anchored to the inner leaflet of the membrane and associated with "raft" lipids by two motifs contained in their C-terminal hypervariable domain. The first motif is a C-terminal CAAX motif that undergoes posttranslational modification by sequential farnesylation, proteolysis, and carboxyl methylation. The second motif is comprised of either one or two palmitoylation sites for N-Ras and H-Ras. H- and N-Ras are known to acquire these lipid modifications at ER/the Golgi while transiting through

the exocytic pathway leading to the PM.

REs are endosomes that are responsible for recycling internalized proteins and lipids to the PM. We have recently shown that H- and N-Ras localize intracellularly at REs and that REs act as a way-station along the post-Golgi exocytic pathway to the PM. H-Ras requires two palmitoyl groups for RE targeting. The lack of either or both palmitoyl groups leads to the mislocalization of the mutant proteins to the endoplasmic reticulum, Golgi apparatus, or the PM. Therefore, we demonstrate that palmitoylation directs Ras proteins to the correct intracellular organelles for trafficking and activity.

Molecular mechanism and physiological functions of plant N-glycan degradation

Takeshi Ishimizu (Osaka University)

We found that N,N'-diacetyl chitobiose (GlcNAc l- 4GlcNAc) is a major N-glycan of S-RNase, the glycoprotein associated with the self-incompatibility of flowering plants. This was the first example of a glycoprotein bearing GlcNAcβl-4GlcNAc as an N-glycan. Then, we discovered the enzyme, endo-β-mannosidase (EC 3.1.2.152), that generates GlcNAcβl-4GlcNAc from Manαl-6Manβl-4GlcNAcβl-4GlcNAc. Purification of this enzyme and molecular cloning of its gene revealed that this endoglycosidase was a plant specific enzyme. This enzyme has unique substrate specificity, hydrolyzing the Manβl-4GlcNAc linkage in $(Man)_n$Manαl-6Manβl-4GlcNAcβl- 4GlcNAc (n=0 ~2). These substrates are generated from high-mannose type N-glycans by the action of jack bean α-mannosidase-like enzyme, which prefers to hydrolyze Manαl-3Manβ linkages. Therefore, we presented a proposed degradation pathway of plant N-glycans. It is that endo-β-mannosidase and jack bean α-mannosidase-like enzyme cooperatively hydrolyze high-mannose type N-glycans to N,N'-diacetyl chitobiose in plant cells. The transgenic plants that the expression level of endo-β-mannosidase was reduced to 30 % of a wild-type plant were observed phenotype alternation, indicating that the degradation of plant N-glycans was essential for glycan metabolism as well as plant physiology.

References

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Special Lecture

A mystery of the cell birth solved by organelle division nanomachineries (polyglucan filaments)

Tsunehiro Kuroiwa (Rikkyo University)

The cell cycle has been taken to mean a mitotic cycle that considered only the dynamics of cell nuclei. However, eukaryotic cells contained at least a minimal set of double-membrane-bound compartments; the cell nucleus, mitochondria, and plastids (chloroplasts), and single-membrane-bound compartments; the microbody, the Golgi apparatus, the endoplasmic reticulum and lysosomes. Therefore, the division cycles of double- and single-membrane-bound organelles should be considered in the cell cycle. In spite of their importance, very little information is known about organelle division cycle. In this talk, I describe strategies for identifying the most suitable organism *Cyanidioschyzon merolae* to reveal the organelle division and their cycles, and introduce the case of mitochondria and plastids as samples. The mechanism of plastid division is very similar to that of mitochondrial division. In the first step, the GTPase dynamin molecules in the plastid-dividing (PD) machinery function as cross-bridges that drive the sliding of the 7nm filaments (polyglucan) in the PD machinery. In the second step, the dynamin molecules play a role in pinching off the narrow bridge between daughter plastids during the late phase of plastid division. Finally I deduce the significance of mitochondrial and PD machineries in the origin of eukaryotes.

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Program November 30 (Tuesday), 2010

Greetings from Organizers

Hisashi Narimatsu (AIST)
Yoshifumi Jigami (AIST)

Session 4　New Findings of Glycan Functions (2)

Chair : Koichi Furukawa (Nagoya University)

Immunological disfunction analysis of Lc3Cer synthase (B3bnt5)-deficient mice lacking polylactosamine on glycolipids

Akira Togayachi (AIST)

Polylactosamine is carried on N-, O-glycans, and glycosphingolipids (GSLs). Polylactosamine is considered to be integral components serving as backbones for the carbohydrate structures. We generated β1,3-N-acetylglucosaminyltransferase 5-deficient (B3gnt5$^{-/-}$) mice to investigate the *in vivo* biological functions of polylactosamine on GSLs (i.e. lacto/neolacto-series GSLs). No Lc3Cer synthase activity and no lacto/neolacto-series GSLs were detected in the tissues of these mice. Ganglioside GM1, known as a glycosphingolipid-enriched microdomain (GEM) marker, was found to be upregulated in B3gnt5$^{-/-}$ B cells by flow cytometry and fluorescence microscopy. However, no difference in the amount of GM1 was observed by mass spectrometry and TLC-immunoblotting analysis. These results suggest that structural alteration of GEM occurs in B3gnt5$^{-/-}$ B cells. We examined whether BCR signaling-related proteins had moved into or out of the GEM fraction. In B3gnt5$^{-/-}$ B cells, these molecules were enriched in the GEM fraction. B3gnt5$^{-/-}$ B cells were more sensitive to the induction of intracellular phosphorylation signals on BCR stimulation and proliferated more vigorously than wild-type B cells. These results suggest that lacto/neolacto-series GSLs play an important role in clustering of GEMs and tether specific proteins to the GEMs. These works were supported by New Energy and Industrial Technology Development Organization (NEDO) in Japan.

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C-type lectins in inflammation and immunity

Tatsuro Irimura (The University of Tokyo)

Regulatory functions of C-type lectins for inflammation and immunity are focus of increased attention by both immunologists and glycobiologists. It has been known that macrophages, dendritic cells and natural killer cells express a variety of C-type lectins. They recognize pathogens and altered self according to their specificity and initiate inflammatory and immune responses. It is likely that the outcome of these innate immune responses initiate consequence of events leading to the diverse features of defense, homeostasis, and pathogenesis. Our focus has been macrophage galactose-type calcium-type lectin, MGL/CD301. By the use of *Mgl1*-knockout mice, we investigated several disease models. In *Mgl1*-KO mice, antigen-specific tissue remodeling and chronic inflammation was not induced with azobenzene-arsonate-conjugated acetylated bovine serum albumin. DSS was orally administrated to *Mgl1*-KO and wild-type mice. The KO mice showed significantly severe inflammation in the colon than wild-type mice after administration of DSS. These results clearly indicate that MGL1 plays a significant role in the regulation of inflammation and immunity.

References

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Diabetic suppressor function of "GnT-IVa" in pancreatic β cells

Kazuaki Ohtsubo (Osaka University)

We have previously reported that the *Mgat4a*-encoded N-acetylglucosaminyl-transferase-IVa (GnT-IVa) is required for the production of an N-glycan structure that acts as a ligand for endogenous lectins that maintain the pancreatic cell surface residency of glucose transporter-2 (GLUT2). The genetic disruption of *Mgat4a* or the diminished expression by high-fat diet administration impaired the GLUT2 N-glycosylation and the endogenous lectin bindings that diminished the cell surface residency of GLUT2. The impaired cell surface expression of GLUT2 thereby abolished glucose-stimulated insulin secretion of pancreatic cells and evoked metabolic disorder diagnostic type-2 diabetes. We have further investigated the cellular and physiologic processes regulating Mgat4a gene transcription and found

that the high-fat diet-induced oxidative stress caused nuclear exclusion of transcription factors and subsequently abolished Mgat4a expression. We further engineered transgenic mice expressing *MGAT4A* in pancreatic cells to rescue the GLUT2 glycosylation under high-fat diet administrated conditions. *MGAT4A* transgenic mice receiving high-fat diet retained GLUT2 glycosylation and cell surface expression that sustained glucose-stimulated insulin secretion responses of cells, and maintained systemic glucose homeostasis. These results indicate that the GnT-IVa deficiency is a part of pathogenesis of type-2 diabetes and GnT-IVa can be a novel suppressor of type-2 diabetes.

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Chondroitin sulfate is essential for normal endochondral ossification

Takashi Sato (AIST)

Chondroitin sulfate (CS) is a glycosaminoglycan chain, consisting of repeating disaccharide units of N-acetylgalactosamine and glucuronic acid residues, and plays important roles in development and homeostasis of organs and tissues. To date, six glycosyltransferases were identified to participate to CS chain synthesis in mammalian. CSGalNAcT-1 and CSGalNAcT-2 are β1,4N-acetylgalactosaminyl-transferases having enzyme activity in both initiation and elongation of CS chain synthesis. Here, we generated and analyzed mice which lack CSGalNAcT-1. Csgalnac1$^{-/-}$ mice were viable and fertile, but exhibited slight dwarfism having a shorter humerus and tibia length as compared with wild-type littermates. Bio-chemically, the level of CS in Csgalnac1$^{-/-}$ cartilage was decreased to ~50% that of wild-type, whereas its chain length was similar to wild-type, indicating that CSGalNAcT-1 participates in the CS chain initiation as suggested in the previous study. Histologically, their growth plate contained shorter and slightly disorganized chondrocyte columns with a reduced volume of the extracellular matrix mainly in proliferative layer. These observations suggest that CSGalNAcT-1 is necessary for normal levels of endochondral ossification. This work was performed as a part of the "Medical Glycomics (MG) Project" supported by NEDO.

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JCGG Luncheon Seminar 2 (sponsored by Kyowa Hakko Kirin Co.)

Development of long lasting erythropoiesis stimulation drugs – Anemia treatment drugs with long serum half-life by glycosylation

Nobuo Nagano (Kyowa Hakko Kirin Co.)

Erythropoietin (EPO) is a glycoprotein hormone that stimulates erythropoiesis. Anemia is a common complication of chronic kidney disease (CKD) due to a deficiency in the production of EPO by the kidneys. The sialic acid-containing carbohydrate content of the EPO molecule positively correlates with its serum half-life and erythropoietic activity *in vivo*. Based on the observation, hyperglycosylated recombinant human EPO (rHuEPO) analogues were developed to increase biological activity. Darbepoetin alfa (NESP) contains 5 N-linked carbohydrate chains compared with 3 in rHuEPO. NESP has 3-fold longer serum half-life and greater biological activity *in vivo* in comparison with rHuEPO. This property allows NESP to be administered at an extended dosing interval. Less-frequent dosing has realized many benefits for not only CKD and hemodialysis patients but also paramedical staffs.

Session 5 Development of Glycan Bio-Markers and Clinical Applications

Chair : Shoko Nishihara (Soka University)

Paradigm shift of clinical laboratory tests by development of glyco-biomarker

Yuzuru Ikehara, Hisahi Narimatsu (AIST)

Medical decision-making is an important responsibility of physician, which is based on evidence. Evidence is well supported by clinical laboratory tests such as detecting hormones produced by tumor and materials derived from destructed tissues. On the contrary, clinical laboratory tests can't provide enough information to make decision for diagnosis and treatment for chronic destructive diseases with slow progression, which include liver fibrosis, Alzheimer disease, and atherosclerosis, etc. For the diseases, surgical pathology and diagnostic imaging are

currently applied.

Glycans-modified variants (GMVs) are designated for glycoprotein that has identical protein sequences but for carrying glycans structures, appear/disappear to associate with disease onset and progression, depending on the microenvironment of pathological sites. For example, one GMV associated with liver fibrosis is a good indicator for disease progression of serum hepatitis and make physician enable patients-enrichment with high risks of developing hepatocellular carcinoma. Moreover, another GMVs can predict a presence of cholangiocarcinoma. We consider that clinical laboratory tests detecting GMVs are feasible to use on diagnosis for chronic destructive diseases with slow progression.

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Development of hepatic disease related glyco-biomakers for addressing unmet-medical needs

Yasuto Tanaka, Atsushi Kuno (NEDO)

Hepatitis C infection is a global health problem. The spontaneous viral clearance was observed in approximately 30% individuals of acute infection. In the therapy using a combination of pegylated interferon-α and ribavirin, approximately 50% of chronic hepatitis C patients infected with high viremia of hepatitis C virus infection (HCV) genotype 1 reached sustained viral response. These findings were strongly expected to affect variations of host genome. To reveal genetic effects against viral clearance or treatment response, we applied a genome-wide association study (GWAS) to HCV infection, and found a strong association of IL-28B polymorphisms with viral clearance or final decision of HCV therapy. The significant SNP is useful for prediction prior to treatment because of the strong association with clinical outcome.

Chronic hepatitis caused by HCV infection will result in an increase in the incidence of cirrhosis accompanied by irreversible progression of fibrosis. About 80% of cirrhosis patients contracted HCC within the past 10 years. We thus

developed an accurate lectin-antibody sandwich immunoassay for monitoring the progression of fibrosis. Fibrosis specific glyco-alteration of alpha1-acid glycoprotein was verified to select lectins for evaluation of the fibrosis progression. The designed immunoassay was fully automated and validated using 900 serum samples.
This work was supported by "Medical Glycomics: MG" project in New Energy and Industrial Technology Development Organization (NEDO) in Japan.

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Validation of stem cells by lectin microarray

Akihiro Umezawa (National Center for Child Health and Development)

Stem cells have a capability to self-renew and differentiate into multiple types of cells; and specific markers are available to identify the undifferentiated or differentiated status of a particular stem cell for developmental biology research and cell-based therapy/regenerative medicine. We aimed to define the status of stem cells and the pluripotency of human ES (hES) and iPS cells using a novel molecular methodology, lectin microarray analysis. Undifferentiated hES cells were clearly distinguished from differentiated hES cells after embryoid formation. The pair-wise comparison means based on "false discovery rate" revealed that three lectins -euonymus europaeus lectin (EEL), maackia amurensis lectin I (MAL I), and phyto-haemagglutinin (PHA) (L)- generated maximal values to define undifferentiated and differentiated hES cells. Furthermore, to define a pluripotent stem cell state, we generated a discriminant for the undifferentiated state with pluripotency. The discriminant function based on lectin reactivities was highly accurate for judgment of stem cell pluripotency. These results suggest that glycomic analysis of stem cells leads to a novel comprehensive approach for quality control in cell-based therapy and regenerative medicine.

Development of cell evaluation system using lectin microarray-based cellular glycomics

Hiroaki Tateno (AIST)

All cells in nature are covered with a dense and complex array of glycans. The total glycan repertoire expressed on cells, "the cellular glycome", varies at every level of biological organizations and in response to intrinsic and extrinsic stimuli. Therefore, the cellular glycome is often referred to as "cell face", which reflects cellular condition. In other words, cells could be discriminated in detail by the use of the cellular glycome. To monitor dynamic glycan alterations, we previously developed a high-sensitive glycan profiling technology, called lectin microarray based on an evanescent field-assisted fluorescence detection principle. We have designed analytical protocols for each sample type such as oligosaccharides, tissue sections, cell membrane hydrophobic fractions, and even live cells. For cell pro filing, we have also developed protocols optimized for each organism such as mammals, fungi, and bacteria, and thus now all organisms are target of this technology. Data processing and normalization procedures were also optimized to ensure the proper interpretation of the data. On the basis of these optimizations, we are now focused on the development of the evaluation system of stem cells in term s of their purity (contamination of xenoantigens) and property (pluripotency, differentiation propensity, tumorigenesis) essential for their safety use in regenerative medicine.

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Session 6 Database for Glycoscience

Chair : Hisashi Narimatsu (AIST)

Development of Japanese unique DB

Toshihide Shikanai (AIST), Toshisuke Kawasaki (Ritsumeikan University)

As part of Life Science Integrated Database project by M EXT, we are integrating glycoscience-related databases cooperating with DB owner research institutes and universities. As initial outcomes keyword or glycan structure released across GlycoScience DBs, and next, platform for data exchange at integrated search will be built up toward the final year. Now we are preparing prototype of the integrated

search, developing basic technologies and organizing the information.

Additionally some more DBs are under construction. Resource DB providing resource information required for research, glycoscience protocols online database (GlycoPOD) for researchers specializing in proteomics as well as glycomics (joint research with Ritsumeikan University), glycan synthesis DB supporting how to obtain desired glycans based on standard preparation (joint research with Noguchi Institute), inhibitor DB for the information on glycosyltransferase and glycosidase activity inhibition and glycoprotein, glycan-associated disease DB, and pathogen adherence to carbohydrate DB (PACDB) . These DBs will be released upon completion and incorporated into the integrated DB.

For the future, we are building infrastructure to link with more DBs including areas peripheral to glycoscience and user-friendly, intuitive interface for researchers specializing in broader area.

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Session 7 Development of Technology for Glycan Synthesis and Its Application (2)

Chair : Yasuhiro Hashimoto (Fukushima Medical University)

Exploring the enzymes that release of sugar chains from all species of glycosphingolipids and the discovery of a novel enzyme that participates in the editing of fungal glucosylceramide metabolism

Makoto Ito, Yohei Ishibashi (Kyushu University)

We have explored the enzymes (EGCase) capable of cleaving the sugar chains from all species of glycosphingolipids (GSLs). In this project, we isolated two genes encoding EGC-globo and EGALC which hydrolyze globo-series GSLs and gala-series GSLs, respectively. Recently, an EGCase homologue, tentatively designated EGCase-related protein (EGrP), was found in several fungal genomic databases. To elucidate the function of EGrP, we characterized a recombinant EGrP expressed in *E. coli* BL21 (DE3). Recombinant fungal EGrP was not able to hydrolyze GMla and TGC, which are good substrates for EGCaseII/EGC-globo and EGALC, respectively. Surprisingly, however, EGrP hydrolyzed glucosylceramide (GlcCer), but not galactosylceramide, at neutral pH. The amino acid sequence of EGrP does not share similarity with other GlcCer-degrading enzymes reported so far. Knockout of the EGrP gene in *Cryptococcus neoformans* greatly reduced GlcCer-degrading activity at neutral pH, resulting in the accumulation of intermediates of fungal- specific GlcCer. These

results strongly suggested that EGrP is a novel glucocerebrosidase that participates in the editing of GlcCer metabolism in *C. neoformans*.

Glycan library and its application to analysis of glycan-recognition by Norovirus

Hiromi Ito, Tomomi Kubota (AIST),
Haruko Shirato (National Institute of Infectious Disease)

An oligosaccharide library including a wide variety of structurally defined oligosaccharides has been established by taking advantage of various glycosyl-transferases. The library can be used not only as a standard for several chemical analyses, but also as a resource for exploring biological roles of glycans, some of which function as profile of glycan binding, and that NoV preferred type 1 linkage over type 2 one, the latter being consistent with the note that NoV infection takes place in small intestine on which type 1 oligosaccharides are known to be expressed. Crystal structures of P domain proteins have been solved with blood group antigens such as A antigen, H antigen a receptor for microorganisms. Here we employed the and Le^a antigen. The P domain protein has a common binding site for the galactosyl moiety and three distinct binding sites for the fucoses from three kinds of antigens. Results of ELISA and SPR assay could be successfully explained by gazing the crystal structures.

Closing Remarks

Hisashi Narimatsu (AIST)