The 16th Symposium
of Japan Consortium for Glycobiology and Glycotechnology

Novel Recipes for Glycosceince

November 26-27, 2018 at Ito Memorial Hall in Ito International Research Center, The University of Tokyo (Tokyo)

Program on November 26 (Mon), 2018

Opening Address
Shoko Nishihara (Soka University)

President Address
Tamao Endo (JCGG President)

Session 1　Pathological Aspects of Diseases and Glycans
Chair: Eiji Miyoshi

Advancement of glycan analysis leads to understand the pathogenesis and the progression of diseases
Yuzuru Ikehara (Chiba University)

Stroma means interstitial tissue, space of which includes lymphatic vessels, lymph nodes, blood vessels and fibroid adipose tissue. It plays a supportive role in the function of parenchyma and is a space for inflammatory response and the following tissue repair. Fibrosis is a pathological remodeling in the stroma, which appears in lymphatic edema developed after surgery of breast cancer, postoperative adhesion of abdominal surgery, hepatitis, asthma, and arteriosclerosis. As the causative correlations between the alterations of glycan structures and the progression of fibrosis are found in liver fibrosis, asthma, and arteriosclerosis, accumulating knowledge in glycan structures in the disease will be clues to understanding the mechanism of the pathogenesis.

Focusing on the pathogenesis in the stroma, I promoted collaborative research. The development of a hemostatic device incorporating plasma technology is one of our successes, which is a hemostatic technique that can prevent postoperative scarring (fibrosis). Additionally, I developed a hybrid type InGaAs semiconductor CMOS sensor camera to visualize the spread of fibrosis at macro or mesoscale. Upon use of my imaging system, the presence of adipose tissue and lymph node can be visualized without using contrast agent or fluorescent substance, and the boundary with fibrotic tissue such as pancreatic tissue and fibrosis can be visually confirmed.

In the lecture, we will introduce "clues to understand morbid tissue repair and clues of fibrotic disease solution" that we hope that the evolution of glycosyl analysis will bring about.

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Regulation of neural vascular barrier function by cell membrane molecules

Eiji Ikeda (Yamaguchi University)

Neural vascular barrier is essential for normal functioning of neural cells, and its impairment by pathological stimuli, including hypoxia and inflammation, is known to be a central of pathophysiology accelerating the progression of various intractable neural diseases. Therefore, the molecules involved in pathological impairment of neural vascular barrier could be new therapeutic targets for intractable neural diseases. Morphologically, the neural vascular barrier function is attributed to the formation of complex tight junction network between endothelial cells. Therefore, first, we focused our study on tight junctions formed between endothelial cells, and established the *in vitro* as well as *in vivo* models of neural vascular barrier.

With these models, we demonstrated that pathological disruption of neural

vascular barrier is mediated through the disappearance of claudin-5, an integral molecule for tight junction assembly, from endothelial cell membranes. Furthermore, we were successful in specifying 3 molecules on endothelial cell membranes, which are ADAM12, ADAM17 and basigin, as the essential molecules for pathological disruption of neural vascular barrier. Here, we introduce our recent data regarding the study on neural vascular barrier, with a focus on the availability of the above molecules as therapeutic targets to prevent and/or repair the disrupted barrier.

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Development of specificity-enhanced tumor biomarkers by mass spectrometric analysis of glycoform subtypes

Koji Ueda (The Cancer Institute of Japanese Foundation for Cancer Research)

In the aspect of prostate cancer diagnosis, the serum prostate-specific antigen (PSA) test has significantly contributed to increase of early detection rate and reduction of prostate cancer-related deaths. Indeed, sensitivity of the PSA test is high enough to detect so early stage prostate cancer, however, high false positive rate of that has often led to overdiagnosis and unnecessary invasive needle biopsy.

To improve the specificity and exclude a false positive diagnosis in PSA test, we established energy-resolved oxonium ion monitoring (Erexim)-based in-depth PSA glycoform profiling technology and explored prostate cancer-specific glycosylation subtypes of PSA. From analysis of the training serum set (15 prostate cancer or 15 benign prostate hyperplasia (BPH) patients whose PSA levels were in "gray zone" (4.0- 10.0 ng/ml), 52 PSA glycoforms were quantitatively detected. Importantly, a couple of glycan structures were specifically observed on prostate cancer patients-derived PSA ($p=1.8 \times 10^{-4}$ or 9.6×10^{-4}), by which constructed a novel prostate cancer-specific diagnosis model (PSA G-Index). When the diagnostic power was assessed using an independent validation sample set (15 prostate cancer and 15 BPH patients in the PSA gray zone), an AUC of PSA G-index was 1.00, while that of total PSA or PSA f/T ratio was 0.50 or 0.60, respectively. Thus, PSA G-Index could serve as a definitive secondary screening method to exclude false positive diagnosis in PSA screening.

Session 2　Roles of Glycan Chains in Infection
Industry-Academia Joint Session sponsored by Tokyo Chemical Industry Co.
Chair: Koichi Furukawa

Search for host factors using genome-editing technology and its application to glycan biosynthesis studies
Toshiyuki Yamaji (National Institute of Infectious Diseases)

Shiga toxin (Stx) and subtilase cytotoxin (SubAB) are virulence factors of Enterohemorrhagic Escherichia coli. Both toxins utilize glycans for receptors to enter the target cells. Stx recognizes a glycolipid Gb3, whereas SubAB binds sialoglycans on glycoproteins. These toxins cause cell death after retrograde transport to the endoplasmic reticulum. In genetic screens against the cell death induced by these toxins, the identified factors may be categorized into two groups: one is involved in retrograde trafficking, and the other is involved in receptor biosynthesis. In this study, a lentivirus-based CRISPR library was used for genome-wide screening to identify host cell genes that conferred resistance to these toxins when the gene was disrupted.

(1) Stx-resistance screening

Most of the enriched genes were sphingolipid- and membrane trafficking-related. In particular, almost all of the genes directly involved in Gb3 biosynthesis were highly concentrated in this screening. Besides these enzymes, two new genes were identified that conferred strong resistance to Stx when they were disrupted. Both proteins were required for the step of Gb3 synthesis from lactosylceramide.

(2) Sub AB-resistance screening

Most were sialoglycan- and membrane trafficking-related genes. Interestingly, not only N-glycan synthesized by MGAT1 but also O-glycans synthesized by C1 GalT1 were used as receptors for SubAB.

Glycan-receptor specificity and interspecies transmission of avian influenza viruses
Masatoshi Okamatsu (Hokkaido University)

Influenza viruses bind to sialic acid terminated glycans expressing on the host cell surface as its receptor. Human influenza viruses preferentially bind to sialic acid linked galactose by α2,6 (SAα2,6Gal) and avian influenza viruses bind to SAα2,3Gal receptors. However, previous researches could not clearly explain the relation between receptor binding properties of viruses and its host specificity. In the present study, we focus on modified sialoglycans which have fucosylated or sulfated modification on third terminus of sialoglycans, which seems to play roles for species barrier of human and animal influenza viruses including highly pathogenic avian

influenza viruses. Our result must explain key factors of interspecies transmission of influenza viruses and extend analysis of pandemic potential of viruses.

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Infectious disease pathology and novel influenza vaccine development

Tadaki Suzuki (National Institute of Infectious Disease)

Influenza virus is the etiological agent of seasonal influenza, which is characterized by mild upper respiratory symptoms. On contrast, avian influenza virus infections in humans cause disease ranging from mild upper respiratory tract infection, severe pneumonia, acute respiratory distress syndrome and even death. Although there is a critical difference in their pathology between these influenza virus infections, infection with both viruses in human occurs via the respiratory mucosal epithelium. The most efficient countermeasure against these influenza virus infection is prevention by means of vaccination. However, injective influenza vaccinations result in an increase only in serum antibody levels, but not in mucosal antibody levels, thereby failing to prevent infection. On contrast, a growing body of evidence shows that mucosal antibodies and efficient protection from influenza virus infection can be elicited by intranasal vaccination, suggesting that this method may be more effective than parenteral alternatives. Profound studies of the mucosal antibody response following intranasal vaccination have also elucidated the detailed secretory IgA antibodies. It can be expected that an intranasal influenza vaccine with its safety and efficacy approved for use in a broad range of people is sure to be available in the near future.

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Luncheon Seminar (sponsored by Shimadzu Co.)

Reconsideration of the pretreatment for N-glycans evaluation

Shuichi Nakaya (Shimadzu Co.)

Many protein-based biopharmaceutical products are synthesized in cultured cells derived from eukaryotes. For this reason, the synthesized proteins are mainly glycoproteins which comprise proteins with glycans linked to them. The glycans in these glycoproteins have diverse and complex branching structures. The structure of the glycan is known to affect the function and stability of the glycoprotein. Therefore, if the structure of the glycan in a synthesized glycoprotein changes, due to changes in the culture environment for example, there may be unexpected changes in the function and stability of the glycoprotein itself. This possibility can lead to serious problems in the development and manufacture of biopharmaceutical products, and therefore monitoring whether the glycan structure has changed or not is a primary element in managing quality. It is important to correctly analyze and evaluate changes in glycan structure, but there are a variety of pretreatment methods for glycan analysis and they are not standardized, so the result of analysis of the same glycoprotein may differ if different pretreatment methods are used.

In this luncheon seminar, we introduce the results of comparing some of the pretreatment methods widely used in N-glycan analysis and investigating how they affect the analysis results.

Session 3　Cutting Edge of Technology Accelerating Glycoscience

Chair : Koichi Kato

Toward observation of glycans using cryo-TEM and atmospheric SEM (ASEM)

Chikara Sato (AIST)

Transmission electron microscopy (TEM) single particle analysis (SPA) determines the structures of proteins from their projections. We have developed SPA and successfully determined the structures of purified protein complexes, including ion channels, receptors, cytoskeletons and oxidative stress sensors[1]. Recently, glycan structure of H1V1 Env was successfully analyzed using SPA at 4.2 Å resolution by Lee et al.[2] SPA is expected to be extensively applied for glycan science. For in-solution observation of cells in natural aqueous environment, we have developed Atmospheric Scanning EM (ASEM), realizing observation of water-immersed cells. The resolution of ASEM was 8 nm near SiN membrane. ASEM realizes high throughput immuno-EM of cells without hydrophobic treatment, and makes it

possible to observe intracellular supermolecular-complex formations and glycosylations: axonal segmentation mechanism in primary culture neurons was revealed[3]. Immuno-electron microscopy of megakaryocyte, bacteria, mycoplasma, rubella virus, ES cell and immuno-dendritic cell in aqueous solution were performed using ASEM; fine structures of biofilms formed by bacteria were successfully observed. Using ASEM and SPA, we aim to merge molecular and cellular level studies for comprehensive understanding of cells.

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Cellulase: Cutting edge of technology for understanding its finely tuned molecular mechanism

Kiyohiko Igarashi (The University of Tokyo)

Cellulose is a major component of plant cell wall and the most abundant biomass on earth. Efficient degradation of cellulose makes it possible to produce fuels and chemicals from plant resources for the achievement of Bioeconomy, although biochemical conversion of cellulose by cellulase is quite slow and the reaction becomes a bottleneck of the process. We recently reported the real-time visualization of crystalline cellulose degradation by individual cellulase molecules using a high-speed atomic force microscopy (HS-AFM), having sub-second time resolution and nanometer space resolution. I will summarize possible molecular mechanisms of the processive enzymes and the natural degradation of crystalline cellulose in addition to the recent neutron crystallography to clarify the detailed hydrolytic mechanisms of inverting cellulases.

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Cleavage and formation of glycan bond: Stereoselective glycoside formation and preparation of antibody-drug conjugates

Shino Manabe (RIKEN)

We present here our recent work based on organic chemistry collaborated with computational chemistry and structural biology. We found a very rare endocyclic cleavage in the anomerization reaction of pyranosides with 2,3-trans carbamate group and showed several endocyclic cleavage evidences by trapping linear cation and computational calculations. Furthermore, we have also developed a unique synthetically useful method for stereoselective 1,2-*cis* aminoglycoside formation from 1,2-trans glycosides in a single-step process.

Antibody-drug conjugates (ADCs) are considered as next-generation antibody drugs, and to expand the safety-margin, homogeneous ADCs are required. Since the conjugation site is specific, on conjugation with glycan, homogeneous ADCs can be obtained with a homogeneous glycan structure. By using the combination of endoS and endoS D233Q, N-glycan with the azide group was introduced at Asn297 to trastuzumab in a site-specific manner. A highly potent monomethyl auristatin E with cathepsin cleavable linker was conjugated to yield a homogeneous ADC via the bio-orthogonal reaction. The prepared ADC showed potent cytotoxicity against high Her2-expressing cells such as N-87, OE-19, and SK-BR-3.

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Novel cancer medicines by the development of fluorogenic chemical probes

Yasuteru Urano (The University of Tokyo)

We have succeeded to develop novel fluorogenic probes for various aminopeptidases based on our newly established rational design strategy with intramolecular spirocyclization. For example, gGlu-HMRG, a novel fluorogenic probe for γ-glutamyltranspeptidase (GGT), which is well-known to be upregulated in various cancer cells, was developed. We succeeded to prove that tumor regions in

real clinical specimen of breast cancer patients, even those smaller than 1 mm in size, could be easily discriminated from normal mammary gland tissues within 1-15 min after probe application.

Encouraged by these promising results, we prepared a library of fluorogenic probes composed of more than 400 probes for various aminopeptidases. These probes were applied on fresh biopsy samples from esophageal cancer patients, and we found the enzymatic activity of dipeptidylpeptidase-4 (DPPIV) was upregulated in tumor-positive biopsy samples, but not with tumor-negative biopsy samples. Indeed, cancer region in the resected human fresh specimens was clearly visualized by topically spraying DPPIV-activatable fluorescence probes within 10 min. These findings clearly demonstrated that our fluorogenic probes should be a breakthrough in rapid detection of tumors during endoscopic and surgical procedures.

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Special Lecture

Chair : Kazuo Yamamoto

An approach with mathematical modeling of complex systems to life: Towards Mibyou or preemptive medicine by dynamical network biomarkers

Kazuyuki Aihara (The University of Tokyo)

In this talk, I discuss possibility of an approach with mathematical modeling of complex systems to life systems. First, I review our research on mathematical modeling of complex systems and explain its theoretical platform composed of (1) advanced control theory of complex systems to analyze nonlinear dynamics of complex systems toward control, (2) complex networks theory to analyze network structure of complex systems toward optimization, and (3) nonlinear data analysis and data-driven modeling to analyze big data observed from complex systems and construct models directly from such data toward prediction. Then, I introduce the DNB (Dynamical Network Biomarkers) theory that makes possible Mibyou or preemptive medicine by detecting early warning signals peculiar to pre-disease states just before transitions from healthy states to disease states.

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Tamio Yamakawa Award Lecture
Chair : Naoyuki Taniguchi

Nutrient regulation of signaling and gene expression by O-GlcNAc
Gerald Warren Hart (The University of Georgia)

O-GlcNAcylation cycles on and off thousands of nucleocytoplasmic proteins and has extensive crosstalk with protein phosphorylation. O-GlcNAc is abundant on nearly all proteins involved in transcription, where it regulates gene expression in response to nutrients. O-GlcNAc also regulates the cycling of the TATA-binding (TBP) protein on and off DNA during the transcription cycle.

Targeted, inducible, deletion of the O-GlcNAc Transferase in αCAMKII positive (excitatory) neurons of adult mice results in a morbidly obese mouse with a satiety defect. Thus, O-GlcNAcylation not only serves as a nutrient sensor in all cells, but also is directly involved in appetite regulation. O-GlcNAcylation also plays an important role in the trafficking of the AMPA receptors in neurons and in the development of functional synaptic spines. Recent studies have shown that more than two-thirds of all human protein kinases are modified by O-GlcNAc and all kinases that have been tested are indeed regulated in some way by the sugar. Abnormal O-GlcNAcylation of CAMKII contributes directly to diabetic cardiomyopathy and to arrhythmias associated with diabetes. Prolonged elevation of O-GlcNAc, as occurs in diabetes, contributes directly to diabetic complications and is a major mechanism of glucose toxicity. Targeted over-expression of OGT to the heart causes severe heart failure in mice, which is reversed when they are crossed with mice having OGA over-expressed in their hearts. Drugs that elevate O-GlcNAcylation in the brain, which prevents hyperphosphorylation, appear to be of benefit for the treatment of Alzheimer's disease in animal models. To date, all cancers have elevated O-GlcNAc cycling, which may play a key role in the regulation of metabolism in cancer cells.

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Program on November 27 (Tue), 2018

Session 4　Design and Utilization of Functional Glycans
Chair : Kazunari Akiyoshi

Organic materials with wonderful sugar biofunctionality
Yoshiko Miura (Kyushu University)

Saccharides played important roles in the living systems as biological ligands. Though saccharide-protein interactions are weak in the monomeric saccharide, they are amplified by multivalency. The multivalent saccharide structures are artificially synthesized with glycopolymers, which are polymers with pendant saccharides. The glycopolymers have been investigated as molecular recognition plastic. The glycopolymers are prepared by the synthetic chemistry techniques. The shape, molecular weight, and saccharide densities were controlled by the polymerization. The saccharide protein interactions were affected by the glycopolymer structures. In addition, the glycopolymer structures were tuned based on the nanotechnology. In the case of porous glycopolymers, the glycopolymers were applied into the protein separation device in the continuous flow system. We also prepared the nanogel particles of glycopolymers. The glycopolymer nanogels adsorbed the target protein. The nanogel of sulfated glycopolymers adsorbed the growth factor to inhibit the angiogenesis.

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Cyclodextrins responsible for the development of supramolecular pharmaceutics

Hidetoshi Arima (Kumamoto University)

In my presentation, I highlight the recent advances in drug delivery techniques utilizing cyclodextrins (CyDs), cyclic oligosaccharides consisting of α-1,4-linked α-D-glucopyranose units, for various drugs such as low molecular-weight drugs, peptide-, protein-, gene-, and oligonucleotide-drugs through a formation of supra molecular complexes. In addition, the utilities of CyDs as vaccine adjuvants and active pharmaceutical ingredients (APis) for various diseases are introduced.

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Separation and analysis of chiral compounds using polysaccharide derivatives

Yoshio Okamoto (Nagoya University)

Many drugs are chiral and their pharmaceutical activities are often different between enantiomers. Therefore, the development of enantiomerically pure drugs has frequently been recommended. For this development, the efficient preparative and analytical methods of enantiomers are highly required. Enantioseparation (optical resolution) by HPLC and SFC, which can be used in both preparative and analytical scales, has been attracted much attention in the past decades. The key of this method is the development of chiral stationary phases (CSPs) with a high chiral recognition ability, and many CSPs based on chiral small molecules and polymers with a recognition ability have already been reported. Among these CSPs, phenylcarbamate and benzoate derivatives of polysaccharides, cellulose and amylose, are today most frequently used for enantioseparation of a wide range of chiral compounds. The chiral separation abilities of the polysaccharide derivatives seem much better than other CSPs so far reported. Polysaccharide-based CSPs are prepared by coating the polysaccharide derivatives on silica gel, meaning that some solvents which dissolve or highly swell the polysaccharide derivatives cannot be used as eluents in HPLC. This defect has been overcome through the immobilization of

the polysaccharide derivatives on silica gel by chemical reactions without changing its recognition ability.

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Development of functional rice cultivars through fundamental studies of starch biosynthesis

Naoko Fujita (Akita Prefectural University)

Starches are large polymers of glucose molecules linked by α-1,4 and α-1,6 glucosidic bonds and are an important source of carbohydrates for human nutrition. Starch biosynthesis in plants is catalyzed by at least four enzymes. Starch synthase (SS) elongates α-1,4 glucan chains, ADP-glucose pyrophosphorylase supplies the substrate for SS, branching enzyme forms α-1,6 branch points, and debranching enzyme trims improper branches in amylopectin. Many isozymes encoding different genes exist in higher plants such as rice. We identified mutant rice lines deficient in these isozymes and compared the structure and physicochemical properties of starch in the endosperm of these mutants with those of the wild type. The data were used to establish a model of amylopectin biosynthesis. In addition, we attempted to improve the agricultural traits of the mutant rice lines, which have unique starch properties, through backcrossing with ultra-high yield cultivars. We focused on rice high in resistant starch (RS) for use in special diets and functional foods. Consumption of boiled rice and rice snacks made with high RS rice results in lower blood sugar and insulin levels than those induced by normal rice. The high RS rice line will be registered in 2019.

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Luncheon Seminar (sponsored by Thermo Fisher Scientific Co.)

Thermo Scientific free glycan, glycopeptide and glycoprotein analysis workflow

Kentaro Takahara (Thermo Fischer Scientific Co.)

Separation and characterization of glycosylated proteins is a critical step in understanding their biological function. Thermo Fisher provides a revolutionary portfolio of work flows, instruments and chemistries to meet these challenges. Whether you are studying intact proteins, glycopeptides, or released glycans, we have the solutions to sweeten your analytical experience.

Structure diversity of O-GlcNAc glycan on Notch1 receptor revealed by glycoproteomics approach

Mitsutaka Ogawa and Tetsuya Okajima (Nagoya University)

On Notch1 EGF repeats, O-GlcNAc is transferred by the EGF domain-specific O-GlcNAc transferase (EOGT). Previously, the detection of O-GlcNAc on Notch 1 was conducted by using anti-O-GlcNAc antibody, lectin, and click chemistry tools. However, it was impossible to quantify that, as suggested that multiple O-GlcNAc sites on Notch 1 preclude quantification of O-GlcNAc level at individual O-GlcNAc sites. Recently, we performed semi-quantification of O-GlcNAc levels on Notch1 by glycoproteomics approach using Orbitrap Fusion and Byonic software. Results revealed that O-GlcNAc-Gal-NueAc trisaccharide structure occurs on restricted EGF domains. In addition, O-GlcNAc level and O-GlcNAc glycan structures are highly divergent among modified EGF domains. In this seminar, we will present actual data analyses and the measurement conditions by trial and error.

Session 5　Novel Functions of Glycans

Chair : Ken Kitajima

Characterization of MPIase, a glycolipid essential for the membrane protein integration

Keiko Shimamoto (Bioorganic Research Institute)

Integration of membrane proteins into biomembranes is a vital event in cells. Recently we identified a novel factor named MPIase (membrane protein integrase) that is essential for the membrane protein integration in the inner membrane of *E. coli*. MPIase catalyzes both translocon-dependent and independent protein integration (Fig. 1a). We purified MPIase from the extract of the inner membrane of *E. coli*. Despite its enzyme-like activity, MPIase is a glycolipid whose glycan consists of about 10 trisaccharide (GlcNAc, ManNAcA, and Fuc4NAc) units (Fig. 1b). About 30% of GlcNAc is 6-O-acetylated. The glycan links with the diacylglycerol moiety

through a pyrophosphate linker. We synthesized a minimal unit of MPIase (mini-MPIase-3), performed the structure-activity relationship studies, and analyzed the physicochemical properties of lipid membranes. Based on these results, we proposed a working model for the integration facilitated by MPIase (Fig.2). The glycan part of MPIase captures the substrate protein to prevent aggregation while the lipid moiety makes the membrane more fluid. Then, more hydrophobic interactions between membrane lipids and the substrate protein would facilitate the procession into the membrane.

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Research and development of blue chrysanthemums – Glycosyl residues of anthocyanin responsible for blue color development by copigmentation –

Naonobu Noda (National Agriculture and Food Research Organization)

Anthocyanin pigments, which comprise anthocyanidin glycosides, are responsible for flower color development. Breeding of blue chrysanthemums by conventional methods such as cross breeding is relatively difficult because there are no closely related species with blue flowers. We therefore tried to create blue chrysanthemums through genetic engineering. Because most bluish flowers contain delphinidin-based anthocyanins, we first established a method to transform the *F3'5'H* gene encoding flavonoid 3',5'-hydroxylase, a key enzyme for delphinidin synthesis, into chrysanthemum. In plants where the introduced *F3'5'H* gene functioned well, delphinidin glycosides accumulated almost exclusively with petal anthocyanins, and the resultant flower color was violet. Next, we carried out experiments to try to alter the flower color to pure blue. We found that blue flowers could be obtained by expressing the *A3'5'GT* gene encoding anthocyanin 3',5 '-O -glucosyltransferase in addition to *F3'5'H*. Thus, the flower color of chrysanthemum can be altered to pure blue by O-glucosylation at the 3'- and 5'-positions of the anthocyanin B-ring. The newly synthesized 3',5'-glucosylated anthocyanins play an important role in developing the blue color through intermolecular associations with

endogenous flavone glycosides. In this symposium, we introduce the latest results from molecular breeding of blue flowers and outline future directions in blue chrysanthemum research.

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A novel regulatory mechanism of cell proliferation by chondroitin sulfate chains

Hiroshi Kitagawa (Kobe Pharmaceutical University)

Chondroitin sulfate (CS), a major component of the cartilage and skin extracellular matrix, is reported to play a role in controlling cell proliferation and differentiation. CS chain is a linear polysaccharide consisting of repeating disaccharide units that can be substituted with sulfate groups at various positions, thereby producing characteristic sulfation patterns. Expression quantitative trait loci (eQTL) analysis demonstrated an association of FAM20B gene with skin disorder psoriasis. FAM20B is a kinase responsible for phosphorylation of xylose in the glycosaminoglycan (GAG)-protein linkage region. Our previous report has shown that FAM20B regulates the sulfation patterns of CS chains as well as the total amount of GAG. Thus, we hypothesized that decreased gene expression of FAM20B might cause pathological conditions such as psoriasis by altering the proportion of CS 6-O-sulfation to 4-O-sulfation. Here we show that the sulfation patterns of CS chains are a crucial regulator of proliferation in skin pathology by using C6ST-1 knockout mice lacking the expression of CS 6-O-sulfation.

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Special Lecture
Chair : Shoko Nishihara

Next-generation technology for robust expansion and differentiation of stem cells based on customized extracellular matrices
Kiyotoshi Sekiguchi (Osaka University)

Cell proliferation and differentiation are strictly regulated by cues transmitted from the microenvironment surrounding cells. The extracellular matrix (ECM) is an essential part of the microenvironment, transmitting signals through interactions with cell surface receptors. Basement membrane (BM) is a ubiquitous ECM underlying or surrounding many cell types. Immunohistochemical expression profiling of >40 BM proteins, including all laminin isoforms, in mouse embryos showed that BM compositions become diversified with progression of embryonic development, but the BM in early mouse embryos is composed of a defined set of proteins, with laminin-511 as a major constituent. We previously reported that laminin-511 is a potent adhesive substratum for human pluripotent stem cells (hPSCs) and supports their vigorous proliferation. We also demonstrated that a recombinant fragment containing the integrin-binding site of laminin-511, designated LM511E8, is more potent than intact laminin-511 in promoting adhesion and proliferation of hPSCs. LM511E8 has now been used as the culture substrate to build a stock of induced pluripotent stem cells for regenerative medicine in Japan.

Session 6　Future Opened by in silico Studies
Chair : Yoshiki Yamaguchi

Integrative analysis of transcription factor occupancy at enhancers and disease risk loci in noncoding genomic regions
Shinya Oki (Kyushu University)

We have fully integrated public ChIP-seq and DNase-seq data (n ~ 78,000), including those for 743 human transcription factors (TFs) with 97 million binding sites, transcription factors (TFs) with 97 million binding sites, and have devised a data-mining platform, designated ChIP-Atlas (http://chip-atlas.org), to identify significant TF-genome, TF-gene, and TF-TF interactions. Using this platform, we found that TFs enriched at macrophage or T-cell enhancers also accumulated around risk loci for autoimmune diseases, whereas those enriched at hepatocyte or macrophage enhancers were preferentially detected at loci associated with HDL-cholesterol levels. Of note, we identified "hotspots" around such risk loci that accumulated multiple TFs and are therefore candidates for causal variants. Integrative analysis of public chromatin-profiling data is thus able to identify TFs and tissues associated with heritable disorders.

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A trans-omic reconstruction of regulatory networks for metabolism

Katsuyuki Yugi (RIKEN)

Advances in comprehensive measurement technologies such as the mass spectrometry and the new generation sequencer are providing landscapes of each 'omic' layer. We have been developing 'trans-omic' analysis[1-3] that allows reconstruction of global biochemical pathways that come across multiple omic layers based on multiple omic data with the supports of public databases and software. To elucidate global regulatory pathways of hepatic metabolism by the insulin-dependent signaling, trans-omic analysis that integrates phosphoprotome and metabolome was performed[4]. Consequently, we found that the insulin signal flowed through the trans-omic network involving 13 protein kinases, 26 phosphorylated metabolic enzymes, and 35 allosteric effectors, resulting in quantitative changes in 44 metabolites (Figure 1). Particularly, the 26 phosphorylated metabolic enzymes include 48 novel phosphorylation sites out of 71. This implies that the insulin signal could be transmitted to broader targets than ever thought. Kinetic modeling analysis predicted selective control of a subnetwork around phosphofructokinase by specific phosphorylation and allosteric regulation. Thus, we provide an unbiased method that reconstructs the trans-omic network from phosphoproteome and metabolome data, which will be applicable to other cellular responses.

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Cell modeling : Basics & application

Mariko Okada (Osaka University)

Our laboratory uses mathematical modeling to understand regulatory mechanism of signal transduction network. In signal transduction network, nonlinear response against cellular environment often plays an important role for cell fate commitment. The mathematical model which has been developed in our laboratory allows us to quantitively evaluate efficiency of drug and genetic variation for cellular output. In

this presentation, I would like to introduce our modeling approaches in order to achieve comprehensive understanding of cancer signaling, which we believe this eventually will permit rational drug repositioning based on the principal of cell control for many types of cancer.

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Glycoinformatics research aiming to understand glycan function: from databases to simulation

Kiyoko F. Aoki-Kinoshita (Soka University)

Glycome informatics has come a long way since the first databases were developed a little over ten years ago. Various standards have developed, allowing data to be more easily exchanged and integrated. GlyTouCan played a key role in this integration by assigning unique accession numbers to glycans, including monosaccharide compositions and fragments. During this time, our lab has been developing many tools for analyzing glycans, many of which are available on the Web at RINGS[1]. We developed data mining algorithms for understanding glycan-binding patterns. We have also developed a tool and database of all human N-glycans based on the known glycogenes in human[2]. Based on this work, we have extended our model to Drosophila embryo, and obtained results that matched mass spectrometry data[3]. Most recently, we have established the GlySpace Alliance, consisting of GlyCosmos in Japan, GlyGen in the US, and Glycomics@ExPASy in Europe. Working together, we will continue to develop databases and tools to help support glycoscience research.

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Closing Remarks