The 14th Symposium of Japan Consortium for Glycobiology and Glycotechnology

Transforming Glycoscience: A bridge toward future perspective and their social contribution

November 1-2, 2016 at Sola City Conference Center (Tokyo)

Program November 1 (Tuesday), 2016

Opening Address
Jun Hirabayashi (AIST)

Welcome address
Naoyuki Taniguchi (JCGG President)

On behalf of the Japan Consortium for Glycobiology and Glycotechnology (JCGG) I would like to welcome all of you to the 14th JCGG symposium. This year Advanced Industrial Science and Technology (AIST) is the main organizer and Dr. Jun Hirabayashi (AIST) has kindly accepted the meeting chair and organized this meeting, which is entitled "Transforming Glycoscience: A bridge toward future perspective and their social contribution"

This meeting also aims to develop cooperation and collaboration within and between academia/institutions and industries especially those in local areas. To further achieve this goal, the RIKEN is also one of the co-organizers of this symposium where the JCGG secretariat is located. Very recently AIST and RIKEN developed an agreement which focuses on cooperation in advanced research and development and the mutual exchange and development of skilled personnel as well as foster- ing human resources. We really expect more intense cooperations and collaborations in glycoscience between these two institutions.

Our JCGG is characterized as having its activity rapidly decided by taking action based on discussions between the board of directors or planning committee members. This year it is noteworthy that we have established the Tamio Yamakawa Award, an international award for research excellence by a glycoscientist. This award was established to honor the distinguished career of Prof. Tamio Yamakawa and his outstanding contributions to the field of glycoscience, and the funding for the award was kindly donated from the Sialic Acid Society in Japan (Secretary general, Dr. Haruo Ogura). Dr. Yamakawa is now a member of the Japan Academy and professor emeritus, the University of Tokyo. Last year he received a distinguished life-time achievement award from the International Sialoglyco Conference and was selected as the Bunka Korosha (Person of Cultural Merit) in Japan. He has reached the age

of 95 years and is still very healthy and active. His CV and scientific contributions can be found on page 87. The chair of the selection committee for the award was Dr. Akemi Suzuki whose mentor was Dr. Yamakawa. The committee members consist of three Japanese glycoscientists and four internationally renowned glycoscientists and we have strictly avoided conflicts of interest during selection procedure, and finally Dr. Markus Aebi, ETH, Zurich was selected as the award winner. He will present the award lecture on November 1. In short, he identified oligosaccharyltransferases in the endoplasmic reticulum by using yeast genetic analysis and explored the biosynthesis of N-glycans which are implicated in congenital disorders of glycosylation (CDG). His research then developed methodology for exploring the quality control of glycoproteins. His accomplishments contributed greatly to the glycoscience community worldwide. He also clarified the biosynthetic mechanism for N-glycan biosynthesis from yeast and bacteria to humans and these studies also progressed to the quality control of proteins. Very recently he applied the biosynthetic mechanisms of N-glycans common to humans and bacteria for a bioengineering technique to efficiently produce recombinant N-linked glycoproteins in an *E. coli* system.

In this symposium Dr. Masaru Suematsu, the president of the Japan Agency for Medical Research and Development (AMED) will give a talk. On January 12, 2016, AMED signed a memorandum of cooperation with the NIH in Washington D.C. The agreement covers cooperative research projects, joint seminars, symposia and other scientific meetings, and the exchange of personnel and researchers. AMED expects the agreement to lead to collaboration in areas such as research on rare and undiagnosed diseases. The AMED made a press release and reported that one of the research areas in the cooperative research projects is the field of glycoscience. We understand that both NIH and AMED agreed that research in glycoscience is a subject of great interest in Japan in terms of medical research and actually AMED recently launched a new project regarding glycoscience with the intent of developing innovative glycodrugs. We wish to acknowledge both AMED and NIH who emphasized the significance of glycoscience in medical research. On the other hand NIH launched a so-called "Common fund" last year and again this year for the support of glycoscience in the US (see page 76). This was actually followed by a Proposal from the US National Academies in 2010 entitled "Transforming glycoscience: a Road map to the future" (translated into Japanese by JCGG)

In this symposium, Mr. Takao Kuramochi, Senior Deputy Director General of Center for Research and Development Strategy, Japan Science Technology will present a talk on November 2. He has been involved in developing policy decisions for innovating science and technology for a long time and will provide valuable suggestions to our community.

Three speakers will present talks in the Special lectures series. Dr. Hiroshi Kiyono, an expert in the field of intestinal immunology who reported that the fucosylation

of intestinal bacteria plays a key role in immunology. Dr. Nico Callewaert will give a talk on glycoscience and industrial relations. Dr. John Magnani who identified selectin antagonists which are useful for the treatment of hematopoietic malignancy and solid tumors will also be a presenter.

Our community has been implicated in the so-called Master plans in a large scale by the Japan Science Academy since 2010. Our proposal from the glycocommunity has consistently been selected as one of the likely candidates but, unfortunately, it has not been funded. This year we asked Dr. Kenji Kadomatsu to take care of this issue and in collaboration with many people he developed a proposal entitled "Platform for the Promotion of Integrated Life Science" in which there are three major pillars, namely 1) the widespread use of the techniques and penetration of glycoscientist to broader scientific communities, 2) remarkable development of research techniques of glycoscience and 3) deep insights into and the clarification of glycan functions. This proposal contains more integrated concepts as compared to previously proposed proposals which had been more focused on glycoscience. This proposal was also discussed with many glycoscientists in Japan including the planning committee members of JCGG, including Dr. Tamao Endo (chair), Dr. Shoko Nishihara, Dr. Koichi Kato and other members including Dr. Koichi Furukawa, Dr. Shinichiro Shoda and others.

Regarding international networks and relations, JCGG published several English language books such as "Experimental Glycobiology" and "Glycoscience: Biology and Medicine" (both published by Springer). In addition some of the international meetings initiated from Japan have been held. GlycoT, initiated by myself in 1997, was held in Toronto organized by Dr. James Dennis. In 2018 the meeting will be organized by Dr. Jianxin Gu in Chen Tao, China.

ACGG (Asian Community for Glycoscience and Glycotechnology) initiated by Dr. Hisashi Narimatsu et al., will be held in Wuxi, China this year and was organized by Drs. Jianxin Gu & Xiao-Dong. The Japan-Netherland Joint meeting was held in Holland, and was organized by Drs. Ken Kitajima and Hans Kamerling in Leiden immediately after the Max Planck-RIKEN joint meeting in Berlin. In August this year Dr. Hisashi Narimatsu, Kiyoko Aoki-Kinoshita and Ichigaku Takigawa organized the 6th Charles Warren Workshop in Sapporo. Therefore, international networks in glycoscience community between Japan and other countries are very active in addition to those of JCGG activities.

Finally I wish to express my sincere appreciation to three ministries such as METL, MEXT and MHLW as well as the Mizutani Foundation, the Uehara Foundation and the Terumo Foundation and various industries who supported our symposium. Without their support it would not be possible to host this symposium. I also wish to acknowledge Mr. Keiichi Yoshida, secretary general of JCGG and various members of AIST and RIKEN for their support of this symposium.

Address
Katsunori Matsuoka (AIST)

Challenge to Application of Glycotechnology to Drug Discovery and Regenerative Medicine

Special Lecture 1
Chair: Jun Hirabayashi (AIST)

Exploiting protein N-glycosylation pathways for biopharmaceutical purposes
Nico Callewaert (Ghent University)

Glycan structures characterize the molecular environment immediately outside of all cell types and hence have critical functions in interactions of any cell with its environment (cell-cell, cell-pathogen, cell-molecule). The field of glyco-biotechnology is concerned with understanding and re-engineering of these glycosylation-dominated interactions. In particular, the understanding of the synthetic pathways and functions for eukaryotic N- and O-glycosylation, gained over the past few decades, has enabled the rewiring of these pathways for the benefit of pharmaceutical applications. Based on the conservation of the core pathways between eukaryotes, it has been possible to transfer the efficient synthesis of particular human-specific glycan structures to other eukaryotes such as yeasts and plants. This is enabling the cost-effective production of biopharmaceutical proteins with glycosylation patterns customized to particular therapeutic functionality (e.g. targeting to particular glycan receptors, or customized for particular pharmaco-kinetic behavior). I will illustrate our work with regard to the production of human IgG-like glycosylation patterns in yeast[1)] and the production of mannose-6-phosphate modified lysosomal enzymes for the treatment of human inherited lysosomal storage diseases[2)]. Whereas these earlier synthetic biology endeavors were geared towards copying the synthesis of complex mammalian glycan structures in other eukaryotes, more recently we have generated mammalian cells, plants and yeast in which glycosylation complexity has been reduced to the bare minimum, while still being compatible with eukaryotic cell life and protein productivity. This 'GlycoDelete' technology[3-4)] opens up many new structural biology and biopharmaceutical applications that are currently being explored in our laboratory.

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Session 1　Progress Reports from the Core National Institutes, RIKEN and AIST

Chair: Hisashi Narimatsu (AIST)

Current Glycoproteomics: Development of a method for large-scale identification of glycosylation site-specific glycan heterogeneity

Hiroyuki Kaji (AIST)

Until recently, glycoproteomics has been recognized as an approach to identify many glycoproteins by capturing glycopeptides specifically with affinity-based or glycan- specific chemical reaction-based methods followed by LC/MS analysis of the captured glycopeptides after deglycosylation. The analyses provide a large numbers of glycosylation sites; however, the approach leads to the loss of direct connectivity of glycan and peptide core. To prevent the loss, direct analysis of glycopeptides is essential. Since the glycopeptide is composed of two kinds of biological oligomers (peptide and oligosaccharide) having different levels of the bond stability, the multistep fragmentation (MS^2 or MS^3) is required for their identification. Because ionization efficiency of glycopeptide is intrinsically low, the MS^n analysis results in a further low identification rate of glycopeptides. Therefore, we developed a method to assign both peptide sequence and glycan compositions of glycopeptides using highly accurate mass of the glycopeptides coupled with calculated masses of core peptides and glycans included in the same sample, in an MS^2-independent manner. In this presentation, we would like to introduce the principle of this method and its application for complex protein mixtures.

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Next generation of theranostics on the basis of in vivo glycan recognition – Diversity of glycan recognition revealed by a structural glycobiology approach–

Yoshiki Yamaguchi (RIKEN)

Human zymogen granule protein 16 (ZG16p) is a 16-kDa soluble lectin expressed in pancreas and intestine, and this is the first mammalian lectin to be described with a β-prism fold. ZG16p has been reported to bind both to glycosaminoglycans and mannose. Crystallographic studies of human ZG16p lectin in the presence of selected ligands revealed the mechanism of multiple sugar recognition the nonreducing end of Manαl-3Man and reducing end of Glcβ1-3Glc fitted into the canonical mannose-binding pocket. Solution NMR analysis using ^{15}N-labeled ZG16p defined the heparin-binding region, which is distinct from the mannose-binding pocket. Hence it is possible for ZG16p to bind simultaneously to both types of ligands. Pathogen-related glycan microarray analysis identified phosphatidylinositol mono- and di-mannosides (PIMI and PIM2) as novel ligand candidates of ZG16p. I will focus on the mechanism of multiple sugar recognition of ZG16p lectin.

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Next generation of theranostics on the basis of *in vivo* glycan recognition –Innovative glycoconjugates by *in vivo* pattern recognition–

Katsunori Tanaka (RIKEN)

We developed RIKEN click reaction (6π-azaelectrocyclization) to efficiently conjugate the glycan molecules to the amino groups of biomolecules. A variety of N-glycans were introduced to the albumin and thus homogeneous and heterogeneous N-glycoalbumins with various valency were subsequently injected to the nude mice. Noninvasive whole body fluorescence imaging revealed that the *in vivo* dynamics and the organ- and cell-specific accumulation were significantly

influenced by the structure of glycan molecules. Such patter recognition mechanisms induced by glycoalbumins were applied to developing innovative theranostics molecules.

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Development of lectin application technologies toward industrialization of regenerative medicine

Hiroaki Tateno (AIST)

I will talk about lectin application technologies for the quality control of stem cells such as human induced pluripotent stem cells and mesenchymal stem cells to realize the industrialization of regenerative medicine.

References
I) Tateno H, Saito S, Hiemori K, Kiyoi K, Hasehira K, Toyoda M, Onuma Y, Ito Y, Akutsu H, Hirabayashi J: Alpha2-6 sialylation is a marker of the differentiation potential of human mesenchymal stem cells. Glycobiology 2016 in press
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Toward curing NGLY1-deficiency

Tadashi Suzuki (RIKEN)

The cytoplasmic peptide:N-glycanase (PNGase) is the enzyme widely conserved throughout eukaryotes. It has been shown that this enzyme is involved in the degradation of misfolded/non-functional glycoproteins destined for degradation called ERAD (ER-associated degradation).

In 2012, a patient harboring mutations of PNGase gene (*NGLY1*) was first reported. Symptom of these patients includes developmental delay, multifocus epilepsy, involuntary movement and liver dysfunction. From this report, it is suggested that an unknown physiological function of PNGase could be contribute to human survival. Our recent study showed that in *Ngly1*-KO cells, ERAD process was compromised. Interestingly, not only delayed degradation but also the deglycosylation of a model substrate was observed in this cell. The unexpected deglycosylation was found to be mediated by the cytosolic endo-β-N-acetylglucosminidase (ENGase), encoded by *Engase* gene. Surprisingly, the ERAD dysregulation in *Ngly1*-KO cells was restored by the additional KO of *Engase* gene. Thus, our study underscores the functional importance of Nglyl in the ERAD process and provides a potential mechanism underlying the phenotypic consequences of the human *NGLY1*-deficiency. In this symposium, we will overview our most recent progress on *NGLY1*-research, and also introduce our efforts to develop in vivo ENGase inhibitors.

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Luncheon Seminar (sponsored by Shimadzu Co.)

Quantitation and qualification of glycans and glycopeptides using high-sensitivity triple quadrupole mass spectrometer: the Erexim Application Suite.

Atsuhiko Toyama (Shimadzu Co.)

While recent glycoproteomic analyses have identified various glycan features and glycosylation sites, many are awaiting quantitative validation at the glycopeptide level. To address this issue, we have previously reported the method of using a triple quadrupole mass spectrometer (TQMS) to reproducibly monitor glvcan-derived low molecular weight ions (oxonium ions) to acquire, within milliseconds, a profile of fragmentation patterns at a range of CID collision energies. This, which we termed the energy-resolved oxonium ion monitoring (Erexim) profile, reflects the glycan structure in such a way that isobaric structures can be clearly distinguished and assigned. The method fully exploits high sensitivity and wide dynamic range of TQMS for quantitative validation of pre-identified targets, such as

biomarker candidates. It is also suitable for high-throughput routine analysis of biopharmaceutical products.

Keynote Speech

Chair: Naoyuki Taniguchi (RIKEN)

AMED starts with IRUD: A hope to save undiagnosed patients through glycomics research

Makoto Suematsu (AMED)

In April 2015, AMED was started in order to fast-track medical R&D and to improve a quality of life for people. Among a diversity of different medical researches, AMED has chosen a field of rare and intractable diseases to tackle with a number of obstacles including rigid and inflexible funding systems and Balkanization of mindsets in academia and researchers, and launched Initiative for Rare and Undiagnosed Diseases (IRUD) as the leading project. It aims to spread a concept of global data sharing for saving patients in foreign countries, and microattribution that should be recognized by all project leaders. Solving Balkanization among different sectors in IRUD which participate in medical R&D gives a clue to fast-track implementation of outcomes among all other fields of medical researches. AMED has also joined in International Rare Disease Consortium (IRDiRC) in order to share many experiences in rare disease researches which were accumulated over 45 years in Japan. I would introduce our novel projects with some successful case-matching examples, and present a hope for glycomics research.

Session 2 Glycoscience-Related AMED Project and Translational Research

Chair: Eiji Miyoshi (Osaka University)

Future perspective for next-generation biologics production

Takeshi Omasa (Osaka University)

Mammalian cell lines are important host cells for the industrial production of pharmaceutical proteins owing to their capacity for correct folding, assembly and post-translational modification. Growing demand for therapeutic antibodies promotes the development of technologies for high quality and productivity in Chinese hamster ovary (CHO) cell production systems. Manufacturing Technology Association of Biologics (MAB) is a technology research association developing next-generation production technologies for modern- and next-generation bio-pharmaceuticals using CHO cell production system. MAB was established September, 2013 and includes 26 Companies, 3 Organizations, 1 National Research and Development Agency and 4 Universities.

Bringing together the expertise, MAB aims to establish an industrial technology platform for biopharmaceuticals. In detail, MAB aims towards i) improvement of the upstream process technology for protein production, ii) improvement of the downstream process technology for protein separation and purification, iii) improvement of the quality assessment technology, iv) expansion and improvement of virus-security control technology, v) combining these technologies to promote the optimization of the manufacturing process. Finally, vi) MAB aims to integrate developed technologies and attempts to use them in practice. This MAB activities is now supported by the project focused on developing key technologies for discovering and manufacturing pharmaceuticals used for next-generation treatments and diagnoses (AMED).

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Industry-Academia Cooperation Seminar (sponsored by Seikagaku Co.)

Chair: Shoko Nishihara (Soka University)

Glycobiological approaches for elucidation of joint disease pathogenesis

Norimasa Iwasaki (Hokkaido University)

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Tamio Yamakawa Award Lecture

Chair: Akemi Suzuki (Tohoku Pharmaceutical University)

The making of N-glycoproteins

Markus Aebi (ETH)

N-linked protein glycosylation is the most frequent post-translation modification in eukaryotic cells. The essential process initiates in the Endoplasmic Reticulum (ER), where an oligosaccharide is assembled on the lipid carrier, dolichylpyrophosphate. Oligosaccharyltransferase transfers the oligosaccharide to selected AsN-X-Ser/Thr sequons (X = any amino acid residue except praline) of polypeptides that enter the lumen of the ER. N-linked glycans then serve as signaling molecules that display the folding status of the attached protein. This signal directs the folding and quality control machinery to the client glycoproteins. Properly folded secretory glycoproteins are directed towards the Golgi compartment where trimming and remodeling of N-linked glycans occurs in a species-, protein- and site-specific manner.

Based on experimental approaches in pro- and eukaryotic model systems, the underlying molecular concepts of the N-glycosylation process and the quality control process in the ER as well as the remodeling of the N-linked glycan in the Golgi will be discussed. It is proposed that in the ER, N-linked glycans are major determinants for the folding of glycoproteins whereas the interaction of the glycan with the folded protein has a strong impact on the remodeling of the N-glycan in the Golgi.

Program November 2 (Wednesday), 2016

Transforming Glycoscience : Interdisciplinary Fusion and Regional Revitalization

Session 3　Potential of Polysaccharides in Material Sciences

Chair: Kazukiyo Kobayashi (Nagoya University)

Promotion of the forum "Great Future of Polysaccharides

Kazukiyo Kobayashi (Nagoya University)

The forum started in 2006 to promote the science and technology of polysaccharides in cooperation with the four Japanese societies: applied glycoscience, cellulose, chitin and chitosan, and cyclodextrins. The main mission of the forum is to explore as yet unknown functions of polysaccharides and to develop highly specialty polysaccharide materials. Polysaccharides and glycoconjugates are the two wheels of glycans. The cooperation of the two wheels is expected to expand the scope of the science and technology of glycans.

Reference

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Molecular and materials design strategy of tough polymers using cyclodextrins

Kozo Ito (The University of Tokyo)

We have recently developed a novel type of polymer network called *slide-ring materials* by cross-linking polyrotaxane, the supra molecular architecture with topological characteristics. In the network, polymer chains are topologically interlocked by figure-of-eight cross-links. Hence, these cross-links can pass along the polymer chains freely to equalize the tension of the threading polymer chains similarly to pulleys. The structure and physical properties of the polymeric materials are drastically different from conventional cross-linked or noncross-linked materials. For instance, the slide-ring gel or elastomer shows quite small Young's modulus, which is not proportional to the cross-linking density and much lower than those of chemical gels with the same density. The concept of the slide-ring gel is not limited to cross-linked gels but also includes elastomer, cross-linked polymeric materials without solvent. Accordingly it can be applied to wide area such as soft contact lens, paints, rubbers, soft actuator and so on. As a typical example, the scratch-resist properties of the self-restoring slide-ring elastomer were adopted into the top coating on the mobile phone.

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Preparation and structures of new cellulose nanofibers, and their applications

Akira Isogai (The University of Tokyo)

The preparation methods of new nanocelluloses and their structures and characteristics are reviewed on the basis of the results primarily obtained in our laboratory. When a catalytic oxidation using TEMPO is applied to fibrous plant celluloses in water at room temperature, significant amounts of sodium carboxylate groups are regioselectively formed on the crystalline cellulose microfibril surfaces. The original cellulose I crystal structure, crystallinity and crystal size are maintained after the oxidation. As a result, complete individualization of crystalline cellulose microfibrils to oxidized cellulose nanofibers with homogeneous ~3 nm widths and high aspect rations of >100 are obtained by gentle mechanical disintegration in water. Various cellulose nanofiber aerogels and forms, and cellulose nanofiber-containing organic and inorganic composites were prepared. Their unique properties associated with some proposed mechanism are introduced in this paper.

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Session 4　Food Glycoscience in Association to Regional Revitalization

Chair: Makoto Ito (Kyusyu University)

Aomori Carbohydrate Research Society and salmon nasal cartilage-derived proteoglycan

Yoji Kato (Hirosaki University)

Proteoglycan (PG) is an important component of animal extracellular matrix as well as collagen and hyaluronic acid, and it is considered to exhibit various functions through interaction with these components and other molecules rather than acting alone. Hirosaki University has promoted studies to apply salmon nasal cartilage-derived PG aiming at activation of the community and contribution to industrial development within it. Recently, many products utilizing PG (food products and cosmetics) have finally been commercialized and these are now sold widely. In this lecture, activities of the 'Aomori Carbohydrate Research Society (inaugurated in 1977)', which served as a parent organization of PG research project at the beginning, are introduced, and applied research on salmon nasal cartilage-derived PG performed by Hirosaki University and in the Hirosaki area with support by the Ministry of Education, Culture, Sports, Science and Technology is outlined. In addition, among these activities, examples of joint research between industries and universities which led to commercialization are introduced.

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Venture into new business by carbohydrate bioengineering

Takashi Kuriki (Glico Co)

Our business was started from scratch, based on our research on carbohydrates in 2002. I will focus my talk on the three important lessons I have learnt throughout the time of my life. The first one was in l 980-90's, from when I was a young scientist

in this field. Second, was from when I was an intrapreneur in 2000's. And lastly, a lesson learnt from 2006, when I became the top executive of this business. The first lesson is how to strike the balance between "selection and concentration" and "diversity". We have to launch products that are diverse, but at the same time, we also have to focus on our core competence. The second lesson is to be adaptive, and to have the mindset of not to become afraid of innovative changes. Our company's research department has adopted this value and has been running under the spirit of "Change or die". The last lesson is to understand the three key points in research and business; "management", "tactics", and "strategy". In addition, designing strategies on a decade-basis and backcasting to achieve desirable goals are equally important.

Industry-academia-government collaboration for rare sugar innovation

Masaaki Tokuda (Kagawa University)

Rare sugars (RS) consist of more than 50 kinds of monosaccharides and their derivatives which rarely exist in nature. Kagawa University discovered the method of RS which allowed us to accomplish functional study of RS. We have already found unique functions in some RS, such as anti-diabetic and anti-obesity effects of D-psicose and anti-oxidative effect of D-allose. Industry-academia-government collaboration (IAGC) of RS has been cooperatively operated in these 15 years. "The Kishoto Seisan Kenko Bukai" is the IAGC team and has led the innovation of D-psicose for functional sweetener and 3 venture companies have been created. IAGC was featured by the formation of "Kagawa Kishoto Senryaku Kaigi" which tries to harmonize the economic strategy of Kagawa Prefecture.

Luncheon Seminar (sponsored by Sumitomo Bakelite Co.)

Total cellular glycomics as unique cellular descriptors

Junichi Furukawa (Hokkaido University)

Cell surfaces are coated with a dense glycocalyx composed of a variety of glycoconjugates such glycoproteins, glycoshingolipids, and proteoglycans. These glycoconjugates play important roles in various biological events on the cell. Many studies report structural analyses of cellular glycans in specific glycoconjugate classes such as N-glycans; however, total cellular analyses showing the relationship between different classes glycoconjugates are scarce. Aiming to elucidate expression profiles of glycans derived from all the major classes of glycoconjugates, we have been developing a series of methodologies for the analysis of various classes of glycoconjugates (N-glycans, O-glycans, GSL-glycans, glycosamino-glycans and free oligosaccharides). These procedures were then combined to v1isualize the entire complement of sugars in the cellular glycome, so-called “total

cellular glycomics". Here, I will first describe the methodologies to realize total cellular glycomics based on glycoblotting procedure[1] and β-elimination in the presence of pyrazolone analogues[2]. Then its application of these methodologies to characterize and classify cells and to identify the cellular biomarker based on extensive glycomic analyses of 18 types of human cells will be presented[3]. Furthermore, we will introduce the applications of total glycomics approach for disease-related biomarkers.

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Special Lecture 2

Chair: Koichi Fukase (Osaka University)

Regulation of epithelial glycosylation by mucosal immunity and gut microbiota

Hiroshi Kiyono and Yoshiyuki Goto (The University of Tokyo)

The gastrointestinal tract is a unique organ which is constitutively exposed to countless numbers of antigens. In order to exclude pathogenic microorganisms and create symbiotic relationships with non- pathogenic commensal bacteria, the host prepares a sophisticated gut immune system including intestinal epithelial cells (ECs). Intestinal ECs apically express α1,2-fucose, which is one of symbiotic molecules to some of commensal bacteria. In this study, we examined the detailed mechanisms of the induction and regulation of α1,2-fucose on intestinal ECs. We identified that segmented filamentous bacteria (SFB}, a kind of commensal bacteria, and IL-22- and lymphotoxin-producing group 3 innate lymphoid cells (ILC3) cooperatively induce epithelial α1,2-fucose. In contrast, CD4 T cells negatively regulate epithelial α1,2-fucosylation by producing IL-10. Therefore, induction and regulation of α1,2-fucosylation of intestinal ECs are controlled by the both arms of innate and adaptive gut immune systems. We also identified that α1,2-fucose on ECs plays a role in protection against infection by pathogenic bacteria such as *Salmonella typhimurium*. These results unveil a novel function of the gut immune

system for the establishment of symbiotic relationship to commensal bacteria and protective platform against pathogenic microorganisms which are mediated by intestinal epithelial glycosylation.

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Industry-Academia Cooperation Seminar (sponsored by Tokyo Chemical Industry Co.)

Chair: Yukishige Ito (RIKEN)

Molecular strategy to control agricultural damage by striga

Shinya Hagiwara (Nagoya University),

What can be realized by chemical synthesis

Ichiro Matsuo (Gunma University)

Special Program

Chair: Kenji Kadomatsu (Nagoya University)

Science and Technology Policy in the great transforming era

Takao Kuramochi (CRDS)

The 5th Science and Technology Basic Plan set out the realization of a world-leading super-smart society (Society 5.0) as a future direction of social transformation. Recent rapid progress of science and technology has opened a door of a completely new society where physical world and cyber world are increasingly merged. A great social change is driven by the paradigm-shift of science and technology. Processes of knowledge-building and value creation have been changed remarkably. STI eco-system needs to become adapted to this big change. Both

academia and industry need to be changed with a view to promoting more comprehensive collaboration. Regarding R&D advancing measures, solution-oriented approach can be effective to develop the STI eco-system and encourage interdisciplinary research. This approach contributes to the co-creation of innovation involving various stakeholders. It has to be developed based on deep understanding of social problems and progresses in science. STI policy should put priorities in strengthening, among other things, STI fundamentals such as human resources, R&D facilities, and funding systems. Since individual policy measures regarding STI infrastructure are closely interrelated each other, they must be well coordinated. Such efforts require the capacity-building of STI policy authorities to develop STI strategies and effective measures to remove obstacles. Closer dialogue and cooperation between research community and policy authorities is essential. Research communities should solve silo-problems and contribute to advance current evaluation systems.

Master plan of glycoscience research in Japan
Kenji Kadomatsu (Nagoya University)

Research activities in the institutes of National Research and Development Agency
Shigeo Koyasu (RIKEN)

Special Lecture 3
Chair: Tamao Endo (Tokyo Metropolitan Institute of Gerontology)

The design, evaluation and clinical development of novel glycomimetic drugs for inflammatory disease and cancer
John Magnani (GlycoMimetics)

All cells in Nature are coated with carbohydrates and these carbohydrates contain structural information that is used in recognition processes among cells, cells and functional proteins, as well as with pathogens and their products. Carbohydrates, however, typically lack drug-like properties (bioavailability, stability, serum half-life, etc). Our strategy is to rationally design highly active drugs based on the bioactive conformation of functional carbohydrates which we call glycomimetic drugs. The first drug candidate known as Rivipansel is a pan-selectin antagonist that inhibits E, P and L-selectins and demonstrates potent activity in pre-clinical animal models of vaso-occlusive crisis in sickle cell disease. Rivipansel not only blocks cell adhesion but also blocks cell activation through β2 integrins at IC_{50} values tenfold lower than

cell adhesion thereby preventing and breaking up occlusions in the bloodstream of sickle cell mice in crisis. Phase II clinical studies were successfully completed in 22 hospital sites in the United States and Canada that tested the use of Rivipansel to treat vaso-occlusive crisis in sickle cell patients. Treatment with Rivipansel resulted in a dramatic and significant reduction in the need for narcotic pain relief as well as a reduction in the duration of the crisis and length of hospital stay. Pfizer has successfully licensed Rivipansel and is now conducting Phase 3 clinical studies worldwide.

GMI-1271 was rationally designed to be a highly specific antagonist only for E-selectin. Using surface plasmon resonance, GMI-1271 demonstrated a KD for E-selectin of 420 nM. In contrast to the normal vasculature, E-selectin is constitutively expressed (3 to 7%) in the bone marrow (BM) microvasculature. As cancer cells circulate through the BM, those cells expressing sialyl $Le^{a/x}$ epitopes bind to E-selectin which activates chemoresistance and immobilization in protective niches. Animal models of acute myelogenous leukemia (AML) demonstrate that leukemic stem cells (functionally determined) in the BM are resistant to the effects of the standard chemotherapy drug, AraC. Either treatment with GMI-1271 or a specific genetic knockout of the E-selectin breaks this chemoresistance and reduces the number of leukemic stem cells in the BM by 90 to 95% (Figure 1). Similar results are seen in another hematologic malignancy, multiple myeloma (MM). The MM cell line, MM1S, was sorted using an antibody HECA452 which identifies the E-selectin ligand, sialyl $Le^{a/x}$. This subclone, $MM1S^{HECA452}$ is highly enriched (>85%) for the expression of the sialyl $Le^{a/x}$ compared to only 5 to 10% expression in the parental MM1S cell line ($MM1S^{par}$). Female SCID beige mice were injected i.v. with either $MM1S^{par}$ or $MM1S^{HECA452}$ ($5x10^5$ cells, n=8/group) and followed for survival. In separate cohorts, the effect of treatment with saline control, GMI-1271, Bortezomib (BTZ) or a combination of both was determined in mice transplanted with either $MM1S^{par}$ or $MM1S^{HECA452}$ cells. Mice transplanted with $MM1S^{HECA452}$ had more aggressive disease with significantly shorter survival compared to those transplanted with $MM1S^{par}$. In contrast to the parental cell line, mice engrafted with $MM1S^{HECA452}$ demonstrated a marked chemoresistance to BTZ treatment. The combination of GMI-1271 and BTZ, however, led to a highly significant improvement in survival of $MM1S^{par}$ engrafted mice (P=0.0363), and more importantly broke the resistance and restored the anti-myeloma activity of BTZ in $MM1S^{HECA452}$ engrafted mice (P=0.0028) (Figure 2). E-selectin, whose expression is increased by chemotherapy, is also known to activate proliferation of normal hematopoietic stem cells in the BM, thereby exposing these normal cells to the side effects of chemotherapy. Treatment with GMI-1271, blocks E-selectin-mediated activation and significantly inhibits the toxic side effects of chemotherapy such as neutropenia, mucositis and death.

GMI-1271 treatment of AML patients has advanced into clinical trials. We conducted a Phase 1 clinical assessment of GMI-1271 in combination with one cycle

of induction chemotherapy (MEC), in heavily pretreated, high-risk AML patients that have failed other therapies (relapsed/refractory). Using GMI-1271 a Complete Remission Rate (CR) of 42% was observed in these failed patients with an Overall Remission Rate (CR/CRi) of 47%. This is higher than expected given the high risk cytogenetic and disease features in this group and the remission duration was sufficient to allow patients to proceed to salvage stem cell transplant (N=S). GMI-1271 is now being advanced into Phase 2 to study the effects on patients at least 60 years of age who have been newly diagnosed with AML and are eligible to receive treatment with the chemotherapy agents cytarabine and idarubicin ('7+3').

GMI-1359 was rationally designed to bind both E-selectin and the chemokine receptor, CXCR4. The BM microenvironment is enriched with cytokines and adhesion molecules, such as SDF-1 (CXCR4 ligand) and E-selectin, which bind AML cells and provide protection against chemotherapeutic agents. In fact, treatment with the standard drug, sorafenib, markedly upregulates CXCR4 expression in FLT3-mutated AML cells. Additionally, leukemia cells activate expression of E-selectin on endothelial cells (EC) that further induce adhesion and chemoresistance within the BM. Based on these *in vitro* results the effects of GMI-1359 combined with chemotherapy was tested in mice engrafted with FLT-3 AML cells. Beginning 14 days post tumor injection, cohorts of mice (n=10/group) were treated with saline, GMI-1359 (40 mg/kg), standard chemotherapy cytarabine plus daunorubicin, or a combination of GMI-1359 and chemotherapy. Combined treatment of mice with GMI-1359 (40 mg/kg) and chemotherapy demonstrated a profound survival benefit compared to controls or chemotherapy alone at day 135 after leukemia cell injection (i.e., 67% vs. 11% or 30 %, p=0.0011 and 0.0406, respectively). As GMI-1359 targets the tumor microenvironment within the BM, it was also tested in solid tumors known to metastasize to the bone such as prostate cancer. Mice were intratibially implanted with luciferase-transfected human prostate cancer cell (PC-3M) for 14 days. The engrafted mice were then treated by intraperitoneal injection for 2 weeks with either saline twice daily; 40 mg/kg GMI-1359 twice daily, 5mg/kg docetaxel once weekly or a combination of GMI-1359 and docetaxel. Thirty-five days after initiation of treatment, the percentage of tibiae positive by X-ray and the size of osteolytic lesions was impacted by treatment with GMI-1359 alone or in combination with docetaxel. Docetaxel alone had only a modest impact on intraosseous lesions. Lytic units were reduced by 38%, 78% and 88% in mice treated with docetaxel alone, GMI-1359 alone, or GMl-1359 in combination with docetaxel, respectively. The significantly reduced intraosseous growth of PC3M cells correlated with decreased serum levels of both mTRAP and type I collagen fragments. Given the significant benefit of combining GMl-1359 with chemotherapy for treatment of both liquid and solid tumors residing in the bone marrow, GMl-1359 was advanced into Phase 1 clinical trials to determine safety and PK in healthy volunteers.

Galectins are carbohydrate-binding proteins and play important roles in

modulating the immune system to affect the progression of cancer. TIM-3 and LAG-3 are two "checkpoints" that down-regulate the immune system by binding to galectins 9 and 3 respectively and limit the ability of the immune system to recognize, respond and destroy cancer cells. GMI-1660 was rationally designed to inhibit both TIM-3 and LAG-3 with IC_{50}'s in the mid-nanomolar range and its use in promoting immunotherapy for cancer treatment is currently under investigation. Galectin-3 in particular also promotes the formation of fibrosis in organs such as lungs, liver and heart after damage. GMI-1570 was rationally designed to potently inhibit Galectin-3 and was tested for inhibiting lung fibrosis induced by damage caused by bleomycin. Seven days after administering bleomycin directly into the lung, mice were treated with GMI-1570 topically at either 10 or 100 μg/mouse once a day for 14 days. GMI-1570 was able to significantly inhibit lung fibrosis as determined by the levels of lung hydroxyproline and histological scoring (Figure 3).

The strategy to develop a new class of potent therapeutic drugs (glycomimetics) based on the bioactive conformations of functional carbohydrates is now being successfully validated by a series of drug candidates described herein that have warranted entering clinical trials by both biotech and large pharmaceutical companies (successfully licensed). This validation identifies the field of Glycobiology as a relatively untapped source of novel therapeutics to address current unmet medical needs.

Closing Remarks