The 5th Symposium
of Japan Consortium for Glycobiology and Glycotechnology

Development of Medical Glyco-Biomarkers and Resolution of Sugar Chain Functions

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Opening Address
Yoshitaka Nagai (President, JCGG)

Invited Lecture (NEDO)
Yoshinori Furukawa

Greetings from Organizer
Hisashi Narimatsu (Organizer, AIST)

Session 1 Development and Clinical Application of Sugar Chain Structure Analysis Technology - (1)

Chairs : Jun Hirabayashi (AIST)
Tatsuro Irimura (The University of Tokyo)

Lectin resource development toward glycan profiling and bio-marker investigation
Jun Hirabayashi (AIST)

Lectin microarray is an emerging technique in glycobiology/glycotechnology, which enables rapid and high-throughput analysis of glycans on the basis of a biological recognition principle. Distinct from the other analytical methods, the method is applicable to not only purified glycans, but also crude samples, such as sera and cell lysates containing glycoproteins. In our developed procedure based on an evanescent-field activated fluorescence detection principle, fluorescently probed glycoproteins derived from cells and tissues are detected in a highly sensitive manner (e.g.<10 ng of glycoprotein or 10^4-10^5 cells) without any washing procedure under equilibrium conditions. More recently, even live cells have become targets for direct profiling by lectin micro array. Apparently, such a method should be a powerful tool for rapid profiling (quality control) of various types of glycoprotein drugs and cells used for immunology and regeneration medicine. The method is also expected to play versatile roles in biomarker investigations that include tumor-related antigens.

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A differential glycan profiling system by means of lectin microarray for glyco-biomarker discovery

Atsushi Kuno (AIST)

Structural changes of glycan are evidently related to disease and other physiological phenomena. Now, glycan is in the limelight as one of the best histological and diagnostic markers. Toward glyco-biomarker development, particular glycoproteins, which have been assigned for "glyco-marker" candidates by the 1st phase, i.e., glyco-biomarker discovery, are generally subjected to enrichment from various clinical samples, and structural diversities of their glycans should be differentially analyzed to relate with disease for the next verification phase. However, to our knowledge, there has been no promising method to analyze glycan structure with a trace amount of an intact protein. Thus, we developed simple, sensitive and quantitative method for glycan analysis by means of antibody-overlay lectin microarray, where a target glycoprotein is enriched by immuno-precipitation followed by direct application on our lectin microarray system without further purification and treatment. Specific signals corresponding to glycans of the target glycoprotein were detected with the aid of an antibody raised against the core protein moiety. The qualit y of this system comes up to high level that enables us to distinguish subtle differences in sialylation frequency and core structure of glycans on proteins at a subpicomole order.

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A fucose-specific lectin from *Aspergillus oryzae* (AOL): a novel probe for detecting core fucose

Kengo Matsumura (Gekkeikan Sake Co. Ltd.)

A probe that specifically detects the αl,6-fucosyl residue ('core fucose') of glycoproteins is important because core fucose is widely distributed in mammalian tissues and is altered under pathological conditions. *Aleuria aurantia* lectin (AAL) and *Lens culimaris* agglutinin-A (LCA) have been often used as carbohydrate probes for core fucose in glycoproteins. Here we show, by using surface plasmon resonance (SPR) analysis, that *Aspergillus oryzae* L-fucose-specific lectin (AOL) has strong preference for the αl ,6-fucosylated pyridylaminated (PA)-sugar chain, suggesting that AOL is a novel probe for detecting core fucose in glycoproteins on the surface of animal cells. A comparison of the carbohydrate-binding specificity of AOL, AAL and LCA by SPR showed that the irreversible binding of AOL to the αl,2-fucosylated PA-sugar chain (H antigen) relative to the αl,6-fucosylated chain was weaker than that of AAL, and that the interactions of AOL and AAL with αl,6-fucosylated glycopeptide (FGP) were similar to their interactions with the αl,6-fucosylated PA-sugar chain. Further more, positive staining of AOL, but not AAL, was completely abolished in the cultured embryo fibroblast (MEF) cells obtained from αl,6-fucosyltransferase (Fut8) knockout mice, as assessed by cytological staining. Thus we conclude that AOL is more suitable for detecting core fucose than AAL or LCA

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A challenge to glyco-biomarker discovery using mass spectrometry

Akihiko Kameyama (AIST)

Glycans have received attention as a target molecule for biomarker discovery since a structural change of glycans is known to be associated with diseases such

as cancer and cell differentiation. Comparative analysis of glycans between malignant and benign samples is necessary for the discovery of glyco-biomarkers. Mass spectrometer (MS), which has become an indispensable tool for the discovery of protein biomarkers, is recently used for glyco-biomarker as well. We introduce a novel approach for glycoproteomics using on-membrane direct MALDI-TOF MS analysis coupled with microdispensing of multiple enzymes onto glycoproteins immobilized on membrane. This approach might be useful for exploring qualitative differences of a particular protein between normal and patient states. For comparative quantification of glycans toward glyco-biomarker discovery, we developed a novel method which uses a glucose oligomer mixture as an internal standard. This method overcomes the inherent difficulty in obtaining reproducible peak intensities by MALDI-TOF MS.

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Large-scale quantitative analysis of glycoproteins for biomarker discovery

Hiroyuki Kaji (AIST)

We paired glycosylation site-specific stable isotope tagging of lectin affinity-captured N-linked glycopeptides with mass spectrometry (Fig. 1), IGOT method[1,2] and determined 1,465 N-glycosylated sites on 829 proteins expressed in *Caenorhabditis elegans*. The analysis shows the diversity of protein glycosylation in eukaryotes in terms of glycosylation sites and oligosaccharide structures attached to polypeptide chams[3]. Currently, we are attempting to develop the method for relative quantitation of the glycopeptide profiles obtained by IGOT method, using 2 kinds of procedure to introduce a stable isotope-tag. One is the PNGase-mediated incorporation of ^{18}O from solvent water to the glycosylated Asn residue of the glycopeptide, resulting in 2 Da difference. Another is the chemical modification for the Lys side chain, i.e. guanidination by O-methyl isourea, resulting in 3 Da difference. To test the procedures, ConA-captured glycopeptide mixture from *C. elegans* extract were divided and labeled differentially, and equal amounts of the mixtures were combined and analyzed by LC-MS shotgun method. We could identified and quantified 527 peptides of 274 proteins at the ratio of 0.80+/-0.14, and 147 peptides of 85 proteins at 0.98+/-0.20 by the respective methods. Thus, both labeling procedures are feasible for the large-scale quantitative analysis of glycopeptide profile for biomarker discovery.

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JCGG Luncheon Seminar 1 (sponsored by Moritex)

Human stem cell glycome profiling using lectin mecroarray

Akihiro Umezawa (National Center for Child Health and Development)

The glycome represents the total set of glycans expressed in a cell. The glycome has been assumed to vary between cell types, stages of development and differentiation, and during malignant transformation. Analysis of the glycome provides a basis for understanding the functions of glycans in these cellular processes. To investigate the glycome of human stem cells, we used the GlycoStation™ system that is a multi-component research instrument designed to measure the profiling patterns of fluorescent signals generated from labeled target glycopeptides and/or glycoproteins hybridized to plant lectin spotted glass microarrays ("Lec-Chip™"). The technique provides a novel strategy for profiling global changes of the human stem cell surface glycome and quality control of donor cells for cell-based therapy.

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Session 2 Invited Lecture: Steps toward International Collaboration - Glycoscience Research in Asia

Chairs : Naoyuki Taniguchi (Osaka University)
Hisashi Narimatsu (AIST)

Glycoscience in Japan: Steps toward International Collaboration

Naoyuki Taniguchi (Japan)

Contribution of Japanese Glycobiologists to Glycoscience

Japan is recognized as one of the most active countries in the field of glycoscience with significant numbers of glycobiologists and glycotechnologists. In

many national and private universities and institutes different projects on glycoscience are ongoing but due to the limited space available in this review, I will focus mostly on national projects in glycobiology involving not just a single group but several groups.

Over the past two decades most of the glycosyltransferases and their genes have been purified/cloned by Japanese scientists. These genes are extensively used in functional glycomics to explore the glycan structure and function.

During the years 2002-2006 the total number of papers published in the section on glycobiology and extracellular matrix section of the Journal of Biological Chemistry (JBC) and Glycobiology, two major journals in the glycobiology field, over 33% were written by Japanese glycobiologists working in Japanese institutions. Since these journals are published by the ASBMB (American Society for Biochemistry and Molecular Biology) and Glycobiology Society of the United States, it is only natural that the majority of the papers are written by contributors working in American Institutions. The percentage of papers by Japanese in the JBC in biochemistry and molecular biology is on average about 10% and in other fields such as signal transduction, genes, and proteins etc. in the journal is around 13%, indicating that the remarkable contribution of Japanese scientists to glycobiology in the field of biochemistry and molecular biology. There is some criticism, however, that in Nature and Science, both high impact journals, very few papers have been published by Japanese glycobiologists working in Japan.

Even though JBC is the world's largest and most cited journal but according to ISI Impact Factor (IF) in 2006, it ranked only 260th among the 6164 scientific journals valuated by IF metrics. The JBC editorial board members recently reported that according to the PageRank algorithm using the Google search engine JBC ranked first, and according to Y-factor which rates journals using equally both IF and Page Rank, the JBC ranked number 6 (ASBMB today July 2007, pages 16-19).

Over half of all known proteins are glycosylated, and glycosylation as one of the most abundant post-translational modification reactions critically affects the functions of proteins. Therefore, structures and functions of glycoproteins have attracted much attentions of scientists in various fields of life science. For characterizing the structures of sugar chains, glycobiology including glycomics has now come of age.

The biosynthesis of sugar chains is catalyzed by glycosyltransferases. Approximately 1% of the total human genome and probably close to 200 to 300 glycosyltransferase genes designated as "glycogenes" exist in humans. A growing body of evidence suggests that the sugar chains in glycoproteins, glycolipids, and proteoglycans play a pivotal role in inciting various diseases such as inflammation, immune diseases, neuromuscular diseases, cancer and metabolic syndromes. We previously proposed the importance of sugar remodeling techniques that involve the production of transgenic mice or knock out mice of the gene(s) or the

knocking down of the gene using siRNA or the overexpression of the gene *in vitro*, in order to identify the target proteins for glycosyltransferases. Moreover, the characterization of functional changes in genes is essential in terms of understanding the real function of sugar chains. Limited data are available at present on KO mice which could identify target protein(s) whose sugar chains are lacking *in vivo*. Even though such KO mice of glycogenes have been developed and if it were possible to observe the phenotypic changes of KO mice, it is not certain what specific types of glycoproteins carrying sugar chains are actually involved in the phenotypic changes and implicated in the processes or symptoms of the disease. Therefore, so-called functional glycomics techniques for the identification of target protein(s) and the characterization of the function of sugar chains play a pivotal role in these studies.

International Networks through Consortia

To further facilitate these efforts several international consortia have now been established. The Consortium for Functional Glycomics (CFG), headed by James Paulson, developed a large research initiative funded by the National Institute of General Medical Science (NIGMS) to understand the role of carbohydrate-protein interactions at the cell surface in cell-cell communication. The CFG aims to define the paradigms by which glycan-binding proteins and their ligands mediate cellular communication. This year CFG has been successfully renewed and will continue for another five years.

The EUROCarbDB design study, headed by Claus-Wilhelm von der Lieth, aims to create the foundations for databases and bioinformatics tools in the realm of glycobiology and glycomics, and has established the principle technical framework for bottom to top initiative where all interested research groups can feed their primary data. The new infrastructure will constitute the nucleus for the creation of a depository of carbohydrate-related data similar to the extensively used data collections in the area of genomics and proteomics. The EUROCabDB organized a training course for bioinformatics entitled Glycobioinformatics and Modeling in Heidelberg this year and more than 100 people including young Japanese scientists participated in this training course which was a great success. The travel expenses for the Japanese participants were supported by the Core-to-Core program funded by JSPS. The Osaka University 21st Center of Excellence Program designated as "Integrated analysis of disease-associated sugar chains and proteins" and the JSPS Core-to-Core program (Leader: Naoyuki Taniguchi) supported the activity of HGPI (Human Disease Glycomics/Proteome Initiative) as described below and continues support mostly to foster young scientists in Japan and international meetings. Actually HGPI held two joint meetings in Florence in 2005 and in 2007 with German glycoscientists including EuroCarbDB.

The Japan Consortium for Glycobiology and Glycotechnolgy (JCGG) was launched four years ago (President: Yoshitaka Nagai). The organization of this consortium is

slightly different from that of the CFG in the U.S. Instead of official support from the Japanese government for its operation, scientists took the initiative for creating and supporting the consortium by using their existing government-supported research grants. Scientists who have been separately funded by different ministries of the Japanese government, such as the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the JSPS, the Japan Science and Technology Agency (JST), the Ministry of Economy, Trade and Industry (METI), the New Energy and Industrial Technology Development Organization (NEDO) and the Ministry of Health, Labour and Welfare are involved in this effort. They have joined together to form this consortium and provide support through individual research grants. The Research Center for Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST) started the Glycogene Project (GG project, research leader: Hisashi Narimatsu) supported by NEDO in April 2001. It is especially noteworthy that among over 185 glycosyltransferases and their genes that have been purified and cloned to date, 62% has been cloned by Japanese researchers. The AIST established newly the Research Center for Medical Glycoscience (RCMG) in November 2006 in order to conduct research which emphasizes the coordination between two research fields, medical science and glyco-engineering supported by METI. The major goal of this project is to develop biomarkers using glycomics (see page 86).

The Riken Frontier Research System on Glycoscience Group was headed by Akemi Suzuki until September 30, 2007. This research team focused on enzymes that are involved in sphingolipid and glycolipid biosynthesis using methods of biochemistry and molecular biology and attempted to modulate the functions of membrane-bound molecules by manipulating sphingolipids and glycolipids. The development of micro-analysis by mass spectrometry for glycolipids and glyco-peptides was also a focus of this research team. Suzuki's group developed the DNA micro array system for glycoscience and opened it for the public. Naoyuki Taniguchi took over Suzuki’s position and started a new project designated the Systems Glycobiology Research Group with three research groups, as Disease Glycomics, Glycometabolome and Structural Glycobiology, on October 1, 2007 by aiming for the fusion of glycoscience with other fields of research such as chemical biology supported by MEXT (see page 100).

The CREST (Core Research for Evolutional Science and Technology) project supported by JST began five years ago and will still continue for another three years. This area includes the clarification of the new functions regarding the roles of sugar chains and mechanisms that control central nervous functions, morphogenesis and differentiation and studies of possible applications such as suppressing cancer invasion and metastasis and the prevention of infection by remodeling sugar chains.

The MEXT project is directed by Koichi Furukawa. A large body of functional glycomics data have now been accumulated mentioned on-going projects and will finish in March 2008 (see page 90).

The four-year-old JCGG organization aims to facilitate the exchange of scientific information, sharing resources, equipment and facilities, constructmg a database, fostermg the development of young scientists, constructing infrastructures, etc. and also aims to construct national research center(s) such as a Systems Glycobiology Center in which glycobiology will merge with other areas such as nanotechnology, bioinformatics and chemical biology in Japan to pursue the above purposes including medical applications within the next three years or so. The JCGG holds an annual symposium, in which each time about 600 people participate (see page 106). Two years ago a joint meeting of JCGG and CFG was also held in Hawaii and over 200 scientists participated and exchanged information.

Under HUPO (Human Proteome Organization), the HGPI (Human Disease Glycomics/Proteome Initiative) was launched and is actively involved in the standardization of mass spectrometry technology for the analysis of sugar chains, and the development of biomarkers for disease. A pilot study among 22 institutions worldwide to analyze standard glycoproteins (human serum IgG and transferring) using mainly mass spectrometry was headed by Yoshinao Wada and was reported this year and now second pilot study for O-glycan analyses is ongoing headed by Anne Dell (U.K.). The next pilot study on biomarker discovery is being planned.

In terms of international collaborations, HUPO plays a key role in the organization of proteomic research but not in glycomics. In 2005 HGPI hosted a joint meeting with German scientists including the EUROCarbDB group in Florence supported by JSPS designated as the Core-to-Core program and in 2006 HGPI will again co-organize a meeting with CFG entitled "Frontiers in Glycomics: Bioinformatics and Biomarkers in Disease" at the main campus of the National Institute of Health NIH), Bethesda (Taniguchi N and Paulson J: Proteomics 7, 1360-6, 2007). At this meeting, the focus group summarized an NIH white paper (see page 94) and emphasized the importance of bioinformatics and biomarker discovery using the glycomics approach. As an outcome of this meeting, the National Cancer Institute (NCI), part of the National Institutes of Health (NIH), is funding a new $15.5 million, five-year initiative to discover, develop, and clinically validate cancer biomarkers by targeting the carbohydrate (glycan) part of a molecule (see http ://www.nih.gov/news/pr/aug2007/nci-2 1.htm).

Recently, in order to facilitate industrial application of glycoscience developed in Japan, AIST and the Research Association for Biotechnology has founded a new type of consortium named GLIT (Glyco-innovation and Industrial Technology). In the future, JCGG and GLIT will work together in order to develop technology for industrial applications based on the fundamental research mostly carried out by JCGG members in academia or other institutions.

Future Perspectives

Since sugar functions are intimately associated with a variety of functions, the present technical approach of glycobiology is limited. In fact most KO mice

experiments were pursued in collaboration with researchers in the fields of behavioral science, psychology, physiology and neuroscience and therefore it is prerequisite for glycobiology to merge with other fields of research such as systems biology, which encompasses the fields of genome, computation, biology and technology and the integration of these techniques will be essential in future research. One of the outcomes and benefits of systems glycobiology will be medical applications of glyco-drugs against inflammatory diseases such as influenza, neuromuscular diseases, metabolic syndromes, cancer and the detection of aberrant glycosylations in the development of tumor markers. Research efforts in Japan as well as international collaboration will also be necessary for significant progress of the biomarker discoveries and their validation.

Glycoscience Research in Taiwan
Chun-Hung Hans Lin (Taiwan)

Initially there was some scattered glycorelated research around in Taiwan for several decades. Over the years, Prof. Albert Wu (who runs a dedicated Glyco-Immunology laboratory at the Medical College of Chang Gung University since 1980s), together with eminent expatriate glycoscientists such as Profs. Yuan C Lee, Yu-The Li, Robert K. Yu, and Chi-Huey Wong, have contributed significantly to promote local glycobiology research. There are two turning points in the development of glycobiology research in Taiwan. First of all, several young glyco-scientists were recruited to the Academia Sinica from 1996 to 1999, along with Profs. Yasuo and Sadako Inoue from Japan, who together catalyzed the more recent phase of glycoscience development in Taiwan. Prof. Chi-Huey Wong, who is himself a renowned carbohydrate chemist and has pioneered several of challenging topics in glycobiology, originally helped to foster the growth but later on has started taking the leadership for the entire development since 2003.

At present, a handful of research laboratories at the Academia Sinica lead a small but vibrant glycoscience community in Taiwan, loosely held together by a monthly chemical glycobiology seminars/forum, an annual Taiwan Glycoscience and Chemical Biology Conference, and several joint research Program Grants. The key participating principal investigators are Drs. Shih-Hsiung Wu, Kay-Hooi Khoo, and Chun-Hung Lin from the Institute of Biological Chemistry, and a few junior colleagues at the Genomics Research Center. In addition, Drs Shang-Cheng Hung and Chun-Chen Lin were previously at the same campus, but moved to National Tsing-Hua University to continue their career at 2005. Most of the current research programs are centered on chemical and structural glycobiology, as well as glycosylation analysis based on mass spectrometry. Recent highlights and focus include development of new methods for the synthesis of complex carbohydrate antigens including modified sialic acids, heparin; preparation of carbohydrate encapsulated

metal nanoparticles and carbohydrate microarrays; structures and functions of glyco-enzymes; development of glycosidase and glycosyltransferase inhibitors; and numerous methodologies and applications in glycomics and glycoproteomics. These distinctively chemistry oriented glycosciences are further boosted by recent integration with the Research Programs of the newly established Genomics Research Center at Academia Sinica in 2003. Dr. CH Wong was the first director of the center. He became the President of Academia Sinica in Oct 2006.

Looking forward to the next phase of development, it is anticipated that the main glycoscience activities will remain focused at the Academia Sinica although satellite laboratories are gradually emerging. Glycomics, based on carbohydrate and carbohydrate-binding protein arrays, as well as mass spectrometry profiling and sequencing, will spearhead most of the technique-driven projects within the next few years to come, with specific applications to glyco-immunobiology in cancer and infectious diseases. In particular, in order to establish a sound platform for tackling important challenges in biology, a special emphasis will be laid on the integration of several components, including synthetic chemistry, structural biology, and other key technology (e.g. new imaging methods and nanotechnology). The leading laboratories will seek global partnership in both the US based Consortium for Functional Glycomics and the Japan based Human Disease Glycomics/Proteomics initiatives. In addition, glycoproteomics is currently a major focus of the National Core Facilities for Proteomics, also located at Academia Sinica. Collectively, grant supports from the Academia Sinica to its participating PIs form the lion share of the financial resources. PIs specializing in glycosciences receive basic institutional funding for their respective laboratories, each further augmented by 1 to 2 joint Program Projects grants. That amounts to about 0.6 million US$ in total from Academia Sinica alone to support glyco-related research, not including additional funding for general analytical facilities. From the National Science Council, another 0.6 million US$ or so in total grant aids can be expected for nation wide glyco-projects which therefore put a rough estimate at about 1.2 million US$ in total funding per annum for glycosciences in Taiwan. Moreover, the government allocates additional substantial budget (called summit projects) to support critical research topics. Fortunately, glycobiology is one of the frontier areas.

Since 1996, several glycoscience-related international meetings generously supported by the government and local workshops have been held in Taipei, at Academia Sinica. Through donation by Profs. Inoue and others, a Foundation for Research and Education of Glycosciences was established in 2001 to promote glycosciences by supporting local meetings and travel for young scientists to participate in international meetings. Affiliated to both the International Carbohydrate and Glyco-
conjugate Organizations, research contributions from Taiwan are now represented in most major international meetings. Personal ties and scientific collaborations

have been fostered with the international glycobiology community and bilateral visits actively cultivated.

Taiwan now enJoys a reasonably strong infrastructure for glycosciences, especially in analysis and synthesis, and is gearing towards more integrated biology under takings. Industrial partnership has been formed with a number of local companies. Support from the Ministry of Economics and the government venture capital to the biotechnology sectors has also been channeled to research and development on several key technologies/products, such as Chinese herbal medicines rich in polysaccharides, the production of recombinant glycoprotein therapeutics, and clinical trials of cancer vaccines. It is anticipated that inputs from individual laboratories engaged in basic glycosciences will bring forth more scientific impact and international visibility as the community attains maturity and critical mass. With Academia Sinica in the driving seat, genomics and proteomics related Program projects are now ushering the basic research into the era of glycomics and glycoproteomics. With active international collaboration and under the strong leadership of Dr. CH Wong, we are confident that glycosciences in Taiwan will continue to gain attention and support from glyco-scientists and funding agencies abroad. The future will very much depend on formation of solid teamwork that can be achieved either in the local community, or by international alliance.

Glycobiology & Glycotechnology in India: Achievements & Future Goals

Avadhesha Surolia (India)

Considering the vastness of India and its established scientific research community, it is a challenging task to justifiably present a global picture of glycoscience in India. However, this abstract will attempt to present a slice of activities in the field of glycobiology and glycotechnology happening in the past decade. Organic chemists in India have greatly contributed to the study of plant-derived gums and polysaccharide structures for more than a century. With the advent of modern '-omics' era, the Indian scientific community has switched to applying fundamental knowledge to the study of challenging biomedical problems.

Pioneering contributions over the past 25 years in the field of structural biology and carbohydrate-binding studies of legume lectins have been made by the laboratory of Surolia, Vijayan, Suguna and co-workers, which formed the basis for mechanistic understanding of the role of lectins in cellular processes (in the absence of immunochemical tools). Their contribution led to the definition of carbohydrate-protein interactions at atomic resolution and the discovery of novel β-prism I and II folds in jacalin and a number of other legume lectins. Studies on thermodynamics have revealed the importance of glycan chains in governing protein folding pathways. Ability of lectins to detect subtle variations in carbohydrate structures has made them paradigms for carbohydrate recognition. Their binding

site is made up of highly conserved loops. However, the molecular basis of as to how a conserved set of residues allow for an overwhelming range of specificities in them was poorly understood. Analyses showed that the remarkable repertoire of legume lectins, for example, emanates from their ability to use a conserved set of residues from loop A and B for hydrogen bonding with a distinct pair of sugar hydroxyls while utilizing variability in other regions (loop C and D) to achieve specificity. Likewise, lectins of moraceae family also follow this paradigm. Moreover, water molecules provide scaffolds as well as contours for carbohydrate recognition while posttranslational modification(s), π-hydroxyl interactions, co-operativity and their oligomerization influence strikingly their specificities. Surolia group has determined the molecular features of carbohydrate recognition by calreticulin, the ER chaperone involved in glycoprotein quality control. Likewise, their studies have recently shown that covalently linked carbohydrates in glycoproteins dictate their folding pathway and behaviour. The role of multivalency and co-operativity in glycan-lectin binding has been studied by Jayaraman and Surolia groups, using photo-active multi-valent synthetic glycodendrimer-based ligands. The strong lectin community in India is highlighted by the works of Swamy, Siva Kumar, Khan and coworkers on conconavalin A, pea lectin, snake gourd lectin, P-type lectins, animal glycosidases, marine and fungal lectins and lectins derived from dengue virus.

Clinical importance of 9-0-acetylsialic acids in acute lymphoblastic leukaemia (ALL) and visceral leishmaniasis (VL) has been shown by Mandal and coworkers using achatinin-H, a novel lectin, and have led to the development of an antigen based diagnosis kit for VL. Studies on clinical samples of rheumatoid arthritis (RA) patients from Indian populations by Hasi Das and coworkers have revealed polymorphism, altered expression and aberrant glycoforms of key signalling bio-molecules. Mayor and coworkers have made significant contributions to understanding of fundamental cellular processes and the role of lipid rafts and glycolipids in Golgi transport and targeting. Molecular mimicry of carbohydrate ligands using peptides and porphyrins and its relevance to constantly changing antigenic epitopes and vaccine design is pioneered by Salunke and co-workers. Studies on structural elucidation of V. parahaemolyticus O3K6 polysaccharide antigen and discovery of unusual monosaccharide components have been achieved by Sen and co-workers using NMR, MALDI-TOF and ESI-MS techniques. Extensive efforts in oligosaccharide synthesis have been made by the groups of Roy and coworkers for synthetic conjugate vaccine development against S. dysenteriae type 4 and S and by Gurjar and co-workers against M. Tb using arabinogalactans. Development of synthetic methodologies, carbohydrate-based small-molecule inhibitor design and crystal structure are pioneered by the groups of Chandrasekaran, Mehta, Ramana, Lognanathan and Vankar.

In the field of gene therapy, Sarkar and coworkers have utilized artificial DNA-loaded F-virosomes to target asialoglycoprotein receptors (AS-GPR) for targeted

gene delivery to hepatocytes. Chaudhury and coworkers have designed ionic synthetic glycolipids as novel agents for effective gene transfection. Studies on bioinformatics and molecular dynamics simulation of substrate binding to sialyl- and other glycosyl-transferases have been accomplished by Balaji, Mohanty and coworkers.

Few frontier areas, such as therapeutic glycoprotein production and biomarker development and validation, however, do need intense focus and development from both academic and industrial community. Sporadic efforts in vaccine and drug development have not materialized from bench-to-bedside highlighting the need for a concerted interdisciplinary effort in basic, clinical and industrial research. There is also an urgent need to introduce glycobiology to undergraduate curricula to nurture future scientists. Currently, new young faculties, such as Sampathkumar and coworkers are focused in the areas of stem cell glycomics, role of glyco-conjugates in the definition of cell types, particularly with regard to stem cell lines generated in India. In collaboration with established proteomics facility at premier centres, efforts are afoot to initiate programs on glycoproteomics.

Indian glycoscience community has been actively participating at international meetings such as annual Glycobiology meetings in USA and Gordon Research Conferences (GRC). Indian Institute of Science (IISc) successfully organized the XVII International Symposium on Glycoconjugates in 2003 and India will be hosting the International Carbohydrate Organization (ICO) symposium in 2014.

With the growing realization and appreciation of the importance of fundamental biomedical research to advances in human health in India and increased funding allocations and thrust in vital infrastructure by government agencies, such as Department of Biotechnology (DBT), Department of Science and Technology (DST), and international resources such as the Wellcome Trust (WT), Human Frontiers in Science Program (HFSP), third world academy of sciences (TWAS), Indo-Japan, and other international collaborative grants, research in glycobiotechnology and glycomics in India is well poised to make great strides and contribute solutions to global health issues in the foreseeable future. Efforts are afoot for the formation of society for glycomics and glycotechnology, to bring scientists from various disciplines under one umbrella, and to press for earmarked funding resources for glycoscience.

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Development of Glycobiology in China

Jianxin Gu (China)

The study of Glycobiology is developing much faster these years in China. More researchers are engaged in this field. Meanwhile, the Ministry of Science and Technology of China, all levels of government and colleges have established funds for special-purpose on glycobioscience to encourage the study on the chemistry and molecular biology of glycoconjugates and their related factors, so that many papers have been published in various journals including Cancer Research, J Biol Chem and Mol Cell Boil.

The National Symposium on Glycobiology and Glycobiotechnology was held in Dalian during Aug 20 to Aug 23, 2006. New members of Glycoconjugates Committee, the Institute of Chinese Biochemistry and Molecular Biochemistry were elected during the meeting. Jianxin Gu, professor and director of Gene Research Center, Shanghai Medical College of Fudan University, was chosen as the new chairman of this society.

Several professors gave meeting reports including "Study on the function and transcription regulation of β1,4-galactosyltransferase V in glioma" by Dr. Jianxin Gu; "The biosynthesis pathway and biological function of mannosylation in filamentous fungi" by Dr. Chen J in; "Recent developments in glycochemistry and glycobiology" by Dr. Peng Wang; "The function mechanism of glypican-1 and syndecan-1" by Dr. Kai Ding; and "The chemical synthesis of oligosaccharides and the discovery of glycol-drugs" by Dr, Xinshan Ye.

Over 100 representatives from various universities and institutes in China attended this forum and made the reports on their technological research findings. The main topics included three aspects: lectins, glycobiology and glyco-chemistry.

1. Lectins

Dr. Zhengmei Zhu kept doing researches on oligosaccharide Le^x and Le^y antigen and found the fucosylated oligosaccharides in seminal plasma may mainly come from epididymis. Further investigation will be requied to speculate the biological significance of fucosylated oligosaccharides in male reproduction.

In the present study, Dr. Jianxin Gu's group reported that Dectin-1, as a receptor for Hsp60, could bind and uptake Hsp60, and demonstrated that Hsp60 was an endogenous ligand of Dectin-1 and employs Syk to propagate its signals and thus regulate macrophage functions.

Dr. Zhang Jianing revealed the glycosylation of CD147 played an important role in lymph metastasis of liver cancer cell in mouse. And the glycosylation of CD147 may become a new target in anti-tumor drugs study.

2. Glycobiology

Much work has been done to study the function and regulation of glycosyltransferases. Dr. Jianxin Gu's group did much job in galactosyltransferases. They found β1,4-galactosyltransferase V plays critical roles in apoptosis, tumor growth and invasion and regulates cell signalings involving in tumor behaviors, eliciting a new mechanism of glioma development and a new mechanism of tumor stem cell formation. They also revealed the β1,4-galactosyltransferase I is critical in liver cancer development.

Dr. Kai Ding worked on the role of heparan sulfate (HS) chain degradation on heparan sulfate proteoglycan in tumor survival and invasion. They found inhibition of HS degradation by inhibitors of heparanase or/and NOS may cause tumor cell growth arrest and cell apoptosis. Angiogenesis array illustrates that metalloproteinase inhibitor TIMP-2 is upregulated when HS degradation is arrested. Interestingly, HS mimetic sulfated polysaccharide may inhibit tumor angiogenesis through block Id1 expression.

3. Glyco-Chemistry

Saccharides contain many complex structures. Thus, it is quite difficult to synthesize various structures of saccharides through organic chemical methods. Dr. Xinshan Ye developed new methods in saccharides synthesis, such as one-pot multi-step strategy.

Researchers in China are focus on not only the synthesis of saccharides but also the function and structure analysis, especially the discovery of related drugs. Dr. Peng Wang has repeatedly demonstrated that the sugar moiety on some carbohydrate-containing small molecules play an critical role in the bioactivity. Variation of such sugar moiety is a feasible drug development approach for new drug candidates with high bioactivity and low toxicity.

Many polysaccharides were purified from plants by Chinese researchers, such as aqueous seaweed polysaccharide, Hericium erinaceus Pers polysaccharide and Ganodema lucidum polysaccharide. And the functions of these polysaccharides were identified by many Chinese researchers, such as anti-bird flu, anti-bacteria, anti-virus, anti-ROS, and anti-tumor. These results are significant in drug discovery.

More and more scientists are joining us to study the glycobiology in China. As we will see, the research of glycobioscience in China is developing in a stable and hopeful way.

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Recent Activities of "Korea Society for Glycoscience"

Dae-Sil Lee (Korea)

In this lecture, the glycoscience research activities in Korea will be briefly introduced, as well as the activities of Korea Society for Glycoscience. At this time, two national programs were being progressed to study glycoscience and industrial implementation of glycotechnology. Also the government is preparing next step for promoting glycoscience activities, including utilization of renewable bio-resources. And Carbohydrate Study Group (CSG) was launched in 1993 to promote carbohydrate researches and to exchange research ideas. And CSG had continuously organized annual meeting for carbohydrate scientists, for which many foreign scientists in this field were invited for monitoring up-to-date progress of glycoscience and glycotechnology. In 2005, CSG was upgraded to form 'Korea Society for Glycoscience (KSG)' which consolidated the research activities of glycoscience community in Korea.

In our recent study, *Thermus caldophilus* GK24 is an extremothermophile in Japanese hot spring and has industrially important enzymes. In order to have many carbohydrate-related enzyme genes, *Thermus caldophilus* GK24 genome have been completely sequenced through shot-gun analysis, and gap-filled. The genome sequence was assembled and annotated by home-made system (Biopot). And all of genes responsible for carbohydrate-related enzymes were identified. And the comparative genome analysis of Thermus species was able to locate the genomic sequences existing only in *Thermus caldophilus* GK24. For example, a gene cluster including endocellulase gene was confirmed for the first time in Thermus species. And genome-based 'Thermus-derived Carbohydrate Synthetic Network' will be introduced in short, particularly focused on its glycolytic pathway.

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Session 3 Development and Clinical Application of Sugar Chain Structure Analysis Technology - (2)

Chair : Yoshinao Wada (Osaka Medical Center and Research Institute for Maternal and Child Health)

Glyco-biomarker detection by 2DICAL - 2 dimensional image converted analysis of LCMS

Masaya Ono (National Cancer Center)

Introduction: Our newly developed proteome platform 2DICAL (2-Dimensional Image Converted Analysis of LCMS) can compare a large number of clinical samples in the form of LCMS data and can find biomarker candidates from the vast data of proteome (Ono et al., Mol Cell Proteomics, 5:1338). We also developed a new module Glycodetector which can detect the glycosylation change of peptide. We introduce the systems for the glyco-biomarker detection.

Materials and Methods: Four commercial CEAs were prepared to the concentration of 0 fmol, 500 fmol and 5 pmol with 1 pmol albumin. 125 serum samples (40 endometrial cancers, 30 controls, 30 uterine myomas, 25 uterine sarcomas) were pretreated by removing the 12 abundant proteins. They were totally digested by trypsin and LCMS and LCMS/MS data were acquired.

Results: Glycodetector found five N-linked glycosylated peptides and 2DICAL showed the glycosylation difference of the peptides among four CEAs. 2DICAL detected 771 peaks to discriminate endometrial cancers, uterine myomas, uterine sarcomas and controls. Among the 771 peaks, Glycodetector selected 68 peaks as peptides with glycoform and 23 peptides were identified by Mascot search

Conclusion: Glycodetector finds peptides with glycoform and 2DICAL analyzes the glycosylation difference of glycoproteins. Their combination is an effective tool for detecting biomarkers with glycoform.

Construction of glycan library and their applications

Hiromi Ito (AIST)

Glycoproteomics or glycomics has become a major academic and clinical research priority. Although oligosaccharides on glycoconjugates play vital roles in biological processes, the functions of glycoconjugates are little understood at the molecular level, mostly because of a lack of sensitive and high-throughput methods for analyzing the structure and interaction of oligosaccharides. To overcome these problems, technologies such as an observational multistage tandem mass spectral library and arrays for carbohydrate and lectin have been developed. However, a large variety of structurally defined oligosaccharides, that is, an oligosaccharide library, is prerequisite for these technologies. Herein, we describe a method for the efficient construction of a glycopeptide and glycan library by using human recombinant glycosyltransferases. Furthermore, applications of their glycopeptide and glycan library will be discussed.

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16:25-17:40

Session 4 Development of Sugar Chain Function Analysis and Knockout Mice

Chairs : Takashi Muramatsu (Aichi Gakuen university)
Akemi Suzuki (RIKEN)

Generation system for induced mutant mice at Tsukuba University and its contribution for the medical glyco project

Satoru Takahashi (Tsukuba University)

To promote research and education in bio-medical sciences, the Laboratory Animal Resource Center at Tsukuba University manages laboratory equipment and systems for animal experiments and provides supervision including the development, maintenance and supply of bio-resources including induced mutant mice. Especially, our Center provides generation service of transgenic mice and gene-manipulated mice by using homologous recombination. To generate gene-manipulated mice, ES cells that were established from C57BL/6J mice in our center are used. It is a great advantage to use ES cells generated from C57BL/6J mice, because there is no need to backcross to C57BL/6J mice. We contribute to the Medical Glyco project for generating conditional knockout mice about ten glycosyl-

transferase genes identified Glyco Gene project. The ES cells derived from C57BL/6J mice and a basic vector, which contains three loxP sites and FRT flanked neomycin resistance cassette, are used for the generation. I will present the detail about generation system of knockout mice in the Laboratory Animal Resource Center at Tsukuba University.

Polylactosamine on glycoproteins influences basal levels of lymphocyte and macrophage activation

Akira Togayachi (AIST)

Polylactosamine is carried on N-glycans, O-glycans, and glycolipids. We have demonstrated that β1,3-N-acetylglucosaminyltransferase 2 (β3GnT2) is a poly-lactosamine synthase that synthesizes a backbone structure of carbohydrate chains *in vitro*. For investigating functions of polylactosamine, we generated β3GnT2-deficient mice and analyzed immunological responses in the β3GnT2-deficient mice. Glycan analysis demonstrated that the amount of polylactosamine on N-glycans was greatly reduced in the tissues of β3GnT$^{-/-}$ mice. We screened polylactosamine-carried molecules of wild-type mice, and found that polylactosamine was present on CD28 and CD19, both known as immune co-receptors. Polylactosamine levels on these molecules were reduced in the β3GnT$^{-/-}$ mice. β3GnT2$^{-/-}$ T cells were more sensitive to the induction of intracellular Ca^{2+} flux on stimulation with anti-CD3ε/CD28 antibodies and were proliferated more strongly than wild-type T cells. β3GnT2$^{-/-}$ B cells also showed hyperproliferation on BCR stimulation. The β3GnT-2$^{-/-}$ macrophages showed augmented expression of CD14 on their cell surfaces, and an enhanced response to LPS, resulting in the increase of inflammatory cytokine production. These results indicate that polylactosamine on N-glycans is a putative immune regulatory factor, presumably suppressing excessive responses during immune reactions.

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Polylactosamne on glycoprotens influences basal levels of lymphocyte and macrophage activation, Pro Natl Acad Sci USA 2007 in press

Insulin resistance as a membrane microdomain disorder

Jin-ichi Inokuchi (Tohoku Pharmaceutical University)

A growing body of evidence implicates glycosphingolipids including gangliosides in the pathogenesis of insulin resistance. We previously demonstrated that in 3T3-Ll adipocytes in a state of TNF-induced insulin resistance, the inhibition of insulin metabolic signaling was associated with an accumulation of the ganglioside GM3, and, moreover, the pharmacological inhibition of GM3 biosynthesis by the glucosyl-ceramide synthase inhibitor D-PDMP resulted in the nearly complete recovery of TNF-induced suppression of insulin signaling, suggesting a new target for therapy against insulin resistance and type 2 diabetes[1]. We found that elimination of insulin receptor (IR) from the caveolae microdomains was associated with an accumulation of GM3[2]. To insight into the molecular mechanisms on the interactions among IR, caveolin-1 and GM3, we performed live cell studies of real-time lateral interactions among IR, Cav1 and GM3 at the plasma membrane, as well as relevant biochemical studies, which all together provide evidence of the dynamic segregation of IR from caveolae microdomains into GEM during the state of insulin resistance[3] (Fig. 1). In addition, our data substantiate a rationale for designing novel therapies (microdomain ortho-signaling therapy) against type 2 diabetes and related diseases based on inhibition of ganglioside biosynthesis.

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The interaction of MBP with meprins resulted in the inhibition of the proteolytic activity of meprins and the activation of the complement system

Toshisuke Kawasaki (Ritsumeikan University)

Mannan-binding protein (MBP) is a C-type serum lectin known as a host defense factor involved in innate immunity, and recognizes mannose, fucose and N-acetylglucosamine residues. Although some exogenous MBP ligands have been reported, little is known about its endogenous ligands. In the present study, both meprin α and β (meprins) have been identified as novel endogenous MBP ligands. Meprins are highly glycosylated zinc metalloproteases, which are expressed in renal and small intestinal epithelial cells and certain cancer cells, and can cleave

extracellular matrix proteins. The binding of MBP to meprins resulted in marked decreases in the proteolytic activity of meprins. Furthermore, interaction of MBP with meprins induced complement activation via lectin pathway. Our results indicate that MBP is an important regulator both for modulation of the localized meprin proteolytic activity and for initiation of complement activation via the binding of MBP to the N-glycans on meprins. Because meprins are known to be some of the major matrix degrading metalloproteases in the renal and intestinal epithelial cells, MBP may contribute, as a potential therapeutic target , to tumor progression. In contrast, the meprins-binding function of MBP may enhance the proinflammatory effects of meprin accumulation in the renal tubular epithelium involving complement activation, ultimately leading to acute renal injury.

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Program November 27 (Tuesday), 2007

Session 5 Development and Trends of Glycan Related Disease Markers

Chairs : Katsuko Yamashita (Tokyo Institute of Technology)
Eiji Miyoshi (Osaka University)
Yoshifumi Jigami (AIST)

Identification of carbohydrate-targetgin tumor markers for lung cancer using Protein Chip system

Koji Ueda (The University of Tokyo)

We will report our new approach to identify carbohydrate-targeting serum tumor markers based on 3 key technologies; 1) removal of abundant proteins from crude sera, 2) lectin-coupled ProteinChip system, and 3) use of a sialylated glycoprotein-compatible matrix THAP. We used 10 lung adenocarcinoma patients' sera and 10 normal controls in this study, whose clinical backgrounds (age, sex, hepatic/renal function, etc.) were exactly matched. For the improvement of MS detection limit, we purified each serum sample with the immunodepletion HPLC column to remove 14 abundant proteins (constituting 94% of serum protein amount). Then these low-abundant components were sequentially processed on Jacalin- or SNA-lectin chips, followed by direct SELDI-TOF MS analyses. In this case, we selected Jacalin or SNA lectin, recognizing glycoproteins with pan-O-type glycans or Neu5Ac(α2,6)-

Gal/GalNAc structures, respectively. We further applied THAP matrix to quantitatively detect sialylated proteins without decay of neuraminic acid moieties. From this screening, a protein peak with m/z = 9700 could be identified as a candidate carbohydrate-targeting tumor marker, for which significant loss of NeuNAc(α2,6)-Gal/GalNAc structure should be expected. Finally we successfully completed both protein identification of the candidate "HSGP1" and determination of an O-glycosylation site by MALDI-QIT-TOF MS^2 or MS^3 analyses. Further validation experiments revealed that HSGP1 protein had two glycan form (I or ll, in Figure 2). Unlike normal samples, glycan structure I was dominant in lung cancer patients' sera.

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Fucose is a target for novel glyco-tumor markers; a detailed analysis for cancer and fucosylation

Eiji Miyoshi (Osaka University)

Fucosylation is one of the most popular modifications with oligosaccharides on glycoproteins or glycolipid. Many kinds of fucosyltransferases, GDP-fucose synthesis pathway and GDP-fucose transporter involve in regulation of fucosylation. Increased levels of fucosylation have been reported in a number of pathological conditions, including inflammation and cancer. Recently, we found that fucosylated haptoglobin is a novel type of tumor marker for pancreatic cancer. The regulatory mechanism for its production would be complicated. Site-directed analyses of haptoglobin oligosaccharides were performed. One site of the N-glycans has a unique structure of oligosaccharides. Fucosylated haptoglobin exists in bile, suggesting that fucosylation of N-glycans regulates secretion of hepatic glycoproteins into bile ducts. Changes in fucosylation could provide a novel strategy for cancer therapy. In this symposium, clinical significance of fucosylated haptoglobin as a tumor marker and regulatory pathway of fucosylation have been discussed.

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Rediscovery of Epiglycanin/MUC21: a potential diagnostic tool

Tatsuo Irimura (University of Tokyo)

The gene for the human homologue of mouse epiglycanin, a mucin expressed on mammary carcinoma TA3-Ha cells but not TA3-St cells, was identified by homology search to a mouse epiglycanin cDNA fragment identified by representational difference analysis between TA3-Ha and TA3-St cells. The open reading frame of this gene was cloned from human cervical carcinoma ME-180 cells. It consists of a mucin domain with 28 non-identical tandem repeats of 45 nucleotides each corresponding to a threonine/serine-rich peptide, a stem domain, a transmembrane domain, and a cytoplasmic tail. The cloned cDNA with a FLAG sequence was expressed in KS62 cells. A combination of immunoprecipitation with a polyclonal antibody specific for the cytoplasmic tail and Western blotting analysis with anti-FLAG antibody and lectins revealed a mucin-like component as the gene product. Analysis by the use of tissue cDNA libraries indicated that the gene is expressed in lung, large intestine, thymus, and testis among 16 normal tissues tested. The polyclonal antibody specific for a synthetic peptide from the cytoplasmic tail, when tested for its reactivity with normal lung tissues, reacted with epithelia of bronchi and bronchioli but not with alveoli. All of 24 lung adenocarcinomas specimens tested were reactive with the antibody, whereas reactivity was observed with only 2 out of 24 squamous and none out of 24 small cell lung carcinomas. This is a novel transmembrane mucin and designated as MUC21

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Development of tumor markers focused on serum glycosyltranferases

Katsuko Yamashita (Tokyo Institute of Technology)

Development of tumor markers is applicable for screening of early staged tumors, and monitoring of recurrence and therapy. Sugar chain structures of various glycoconjugates change by tumorgenesis and have been using as tumor markers. However, they were not useful for diagnosis of early staged tumors. We focused on serum secreted glycosyltransferases, which are ectopically expressed in tumor cells,

since Golgi-resident glycosyltransferases are not static but dynamically exchangeable. To assess a possibility whether these enzymes serve as novel tumor markers, we prepared monoclonal and polyclonal antibodies against β1-3 galactosyltransferase-5 (β3Gal-T5) and N-acetylglucosamine: 6-0-sulfotransferase-2 (GlcNAc6ST-2) proteins, and constructed a sandwich method to detect the proteins in the sera from ovarian cancer patients. As a consequence, both the enzymes were not detected in the sera from normal women, while increased levels of the enzymes were found in the sera from stage Ia ovarian cancer patients, suggesting that the specific serum glycosyltransferase proteins are applicable as tumor markers of early stages of various cancers.

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Molecular analysis of podoplanin, a platelet aggregation - inducing factor

Yukinari Kato (AIST)

Podoplanin (Aggrus) is a mucin-type sialoglycoprotein that plays a key role in tumor cell-induced platelet aggregation and metastasis. Podoplanin possesses a platelet aggregation-stimulating (PLAG) domain, and Thr52 in the PLAG domain of human podoplanin is important for its activity. We recently identified C-type lectin-like receptor 2 (CLEC-2) as an endogenous receptor of podoplanin on platelets. In this study, endogenous or recombinant human podoplanin were purified using a novel anti-podoplanin antibody (NZ-1), and total glycosylation profiles were surveyed by lectin microarray. Analyses of glycopeptides produced by Edman degradation and mass spectrometry revealed that the disialyl-core1 (NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAcα1-O-Thr) structure was primarily attached to a glycosylation site at residue Thr52. We next synthesized various glycopeptides of podoplanin that includes both PLAG domain and O-glycan on Thr52. Interestingly , a disialyl-core1-attached glycopeptide was specifically recognized by CLEC-2. These results indicated that the disialyl-core1 at Thr52 is critical for podoplanin-induced platelet aggregation. Podoplanin and CLEC-2 might represent a promising therapeutic target in cancer metastasis.

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Production of mucin-type glycoprotein in yeast

Yasunori Chiba (AIST)

N-Glycan remodelings in yeast have been studied in recent years, however the production of a glycoprotein containing a mucin-type glycan in yeast cells has not been reported. We succeeded in engineering yeast strains capable of producing glycopeptides and glycoproteins containing mucin-type glycan, such as MUC1a and human podoplanin. Podoplanin requires mucin-type glycan for platelet aggregation, and recombinant podoplanins produced by the yeasts do possess the activity. In our technology, substitution of introduced glycosyltransferases made possible production of different patterns of glycosylations on a glycoprotein, which is useful to determine the functional O-glycan structure of each glycoprotein. Although a biological role of mucin-type glycan has still remained to be elucidated, creation of a tumor-specific glycopeptide led to produce a novel antibody recognizing a specific marker for a highly aggressive fibrosarcoma cell type. A large amount of glycoprotein containing mucin-type glycan in our system may be useful for developing antibodies against tumor-specific glycopeptide epitopes.

Development and appliation on a novel drug delivery system using the carbohydrate function

Yuzuru Ikehara (AIST)

We developed a new technology for accumulation of anti-cancer drugs at the extranodal lymphoid tissue in the omentum to control intra-abdominal metastatic foci using oligomannose-coated liposome (OML) as a carrier of anti-cancer drugs. This approach is based on the recognition of specific carbohydrates (oligomannose) on liposomes by intraperitoneal macrophages through the specific binding between the carbohydrate and the cell surface receptor, followed by activation of the macrophages. The liposomes coated with oligomannose (OMLs) have led to significant improvement in the specificity and efficiency of uptake by macrophages, and that intracellular hyperthermia - achieved by using magnetic nanoparticles encased in the OMLs and an alternating magnetic field - could control the release of the components carried from the macrophages. To demonstrate the feasibility of using this promising technology in clinical studies, and because we found that intraperitoneal macrophages home to intra-abdominal metastatic sites (in the omentum) after uptake of OMLs, we attempted to use the techniques to control intra-abdominal metastases and succeeded in demonstrating the remission of

tumours in our animal model. My talk will discuss the feasibility of using this technology, and provide an overview of current attempts to use this drug delivery system for intra-abdominal metastases.

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JCGG Luncheon Seminar 2 (sponsored by Shimadzu Co.)

Recent trends on mass spectrometry
Yoshinao Wada (Osaka Medical Center and Research Institute for Maternal and Child Health)

Session 6 Developmental Trend of Glycodatabase
Chair : Toshisuke Kawasaki (Ritsumeikan University)

KEGG as a glycome informatics resource
Kosuke Hashimoto (Kyoto University)

In glycomics, most of the primary data is information concerning either glycan structures or glycan-related genes. To help predict complex cellular processes involving glycans it would be helpful to collect this data into a single resource. With this goal in mind, we have been developing KEGG (Kyoto Encyclopedia of Genes and Genomes) to include three basic glycan resources (1) GLYCAN, a database of glycan structures; (2) the glycosyltransferase and the glycosidase reaction library; and (3) glycan-related pathways

With a complete knowledge of glycosyltransferase reactions, it should be possible in principle to predict the repertoire of glycan structures in an organism from the repertoire of glycosyltransferase sequences found in its genome. We have developed the Composite Structure Map (CSM), a map illustrating all the possible variations at glycan structures within an organism, and have developed an algorithm for the prediction of glycan structures from gene expression and glycosyltransferase reaction data. Using the same principles, we have also been studying fatty acid structures, which also have a huge variation among organisms. We have elucidated

the repertoire of fatty acid modifying desaturases and elongases and predict the fatty acid variations found in 56 organisms

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The portal site of the glycodatabases in Japan
Toshihide Shikanai (AIST)

Session 7　"Great Future of Polysaccharides" Project

Promotion of the project "Great Future of Polysaccharides"
Kazukiyo Kobayashi (Nagoya University)

The Project "Great Future of Polysaccharides" launched in 2006, in cooperation with several polysaccharide-related scientific societies, to exploit renewable polysaccharide resources on earth and explore their potential functionalities. A massive amount of polysaccharides such as cellulose, starch, chitin/chitosan, and others are produced by life on earth under the blessing of the sun. The importance of polysaccharides as foods, materials, and medicines is well recognized. The science and industry of polysaccharides are grappling with the strategies such as sustainable resources, biomass energies, green sustainable chemistry, biodegradable polymers, and bioprocesses, in addition to food and health. Recently, the scope of polysaccharides has expanded to several new types of high-tech materials with quite unique properties characteristic of polysaccharides. The project expects to open the new fields of science and technology of polysaccharides and to send the information of the polysaccharide worlds to modern societies.

Also, polysaccharides can be regarded as one aspect of glycans associated with glycoconjugates inside and outside of living cells. I believe that the Project "Great Future of Polysaccharides" is closely connected to the activity of Japan Consortium for Glycobiology and Glycotechnology. I ask for the JCGG community to understand and support this project.

Session 8　Ministry of Education and Science Grants-in -Aid for Scientific Research "Glycomics" Report

Chairs : Koji Kimata (Aichi Medical University)
Koichi Furukawa (Nagoya University)

Neuronal injuries and keratan sulfate

Kenji Kadomatsu (Nagoya University)

Neurons in the adult central nervous system do not spontaneously regenerate after injuries. Chondroitin sulfate glycosaminoglycan is one of the major inhibitory factors to neuronal axon regeneration. Another glycosaminoglycan, keratan sulfate (KS), is induced after neuronal injuries, but its biological significance has been poorly understood.

We found that mice deficient in N-acetylglucosamine:6-O-sulfotransferase-1 (GlcNAc6ST-1) lack KS in the central nervous system. We employed two injury models, i.e. cortical stab wound and spinal cord contusion injury. Expressions of 5D4-positive KS and GlcNAc6ST-1 were induced after neuronal injuries, but 5D4-positive KS was not detected in the deficient mice even after injuries. These model revealed that KS plays a critical role in inhibition of neuronal axon regeneration.

I will discuss the biological significance of KS in neuronal injuries and its possible application to bed side.

Neural plasticity and the HNK-1 carbohydrate

Syogo Oka (Kyoto University)

In the nervous system, various types of cells recognize and interact with each other to form precise neural network. During these processes, carbohydrates expressed on proteins give their carrier proteins structural diversities, resulting in regulating the cell-cell recognition, interaction and cell migration. Among these carbohydrates, HNK-1 (Human Natural Killer-1) carbohydrate is one of the most characteristic glyco-epitope in the nervous system. To reveal the function of the HNK-1 carbohydrate, we generated gene-deficient mice of GlcAT-P, which is a major glucuronyltransferase in the nervous system. The GlcAT-P gene deficient mice exhibited reduced long-term potentiation (LTP) at the Schaffer collateral-CA1 synapses and defects in spatial memory formation. Therefore, the HNK-1 carbohydrate plays crucial roles in synaptic plasticity. However, little is known about the molecular mechanisms how the H NK-1 carbohydrate controls synaptic plasticity. In this study, we have found the evidence that the HNK-1 carbohydrate is expressed on AMPA receptor and has important roles in spine formation. These results suggest that the HNK-1 carbohydrate on AMPA receptor is involved in spine

formation and in the regulation of synaptic plasticity.

Cleavage-secretion of sialyltransferase - Its formation mechanism and application to hepatitis diagnosis-

Shinobu Kitazume (RIKEN)

Soluble glycosyltransferases are proteolytic cleavage products of membrane-bound glycosyltransferases. The levels of soluble glycosyltansferases are increased in cancer and inflammation, suggesting that the cleavage of glycosyltransferases could be a regulatory mechanism. We previously found, for the first time, that BACE1 is involved in the cleavage and secretion of sialyltransferase[1,2]. BACE1 is a membrane-bound aspartic protease that cleaves amyloid precursor protein to produce a neurotoxic peptide, Aβ, and is implicated in triggering the pathogenesis of Alzheimer disease. Our subsequent and recent finding indicates a novel regulatory mechanism in which cleavage and secretion of ST6Gal1 enhance the sialylation of soluble glycoprotein substrates[3]. Another point of view is that plasma ST6Gal1 may represent a sensitive biomarker to indicate hepatopathological situations, and we developed a sandwich ELISA system to detect plasma ST6Gal1. Interestingly, in Zone 1 or Zone 3 hapatocyte injured rats, and hepatitis patients, the levels of plasma ST6Gal I were significantly higher as compared with those in control sample

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Physiological roles of heparan sulfate remodeling mediated by novel endosulfatases

Kazuko Masu (Tsukuba University)

Heparan sulfate proteoglycans regulate neural differentiation, axon guidance, synaptogenesis, and plasticity. These activities are mediated by the heparan sulfate (HS) chains covalently-attached to the core proteins of proteoglycans. In HS chains, some parts of sugars are epimerized and sulfated at specific residues, generating enormous structural heterogeneities. Specific structures in HS chains are thought to be required for an interaction with signaling molecules and extracellular matrices. Recently, we have isolated two novel members of the sulfatase family, called SulfFP1 and SulfFP2, which are evolutionarily conserved from the nematode and fruit fly to human. We have shown that they have endosulfatase activity catalyzing hydrolysis of 6-O-sulfates in the HS chain. Some *in vitro* studies showed that SulfFPs activate

Wnt signaling, while suppressing FGF signaling. In order to examine *in vivo* function(s) of SulfFPs, we generated knockout mice for each gene. Although single knockout mice did not show apparent abnormalities, double knockout mice died soon after birth. In these mice, fractions of tri-sulfated disaccharide increased, suggesting that the endosulfatase activities of SulfFPs remodels HS *in vivo*, and that these HS modifications are necessary for normal development. We are analyzing neural phenotypes in double knockout.

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The mechanism of heparan sulfate sulfotransferase-3 (HS6ST3) cellular localization

Naoko Nagai (Aichi Medical University)

The biosynthesis including the 6-O-sulfation of the heparan sulfate occurs in the Golgi apparatus. However, the activity of the heparan sulfate 6-O-sulfotransferase (HS6ST) was abundantly found in the supernatant of the culture medium. We have investigated the secretion mechanism of HS6ST3, one of the HS6ST isoform by using the stable cell line of CHO which expresses GFP-tagged HS6ST3-GFP. We showed that the HS6ST3-GFP became soluble protein in the early phase of the secretory pathway by Triton X-114 phase partition experiment. When we treated the cells with the cell-permeable β3-secretase inhibitor, Z-VLL-CHO, the secretion was inhibited and HS6ST3-GFP accumulated in the cell HS6ST3-GFP co-localized with the endoplasmic reticulum marker, POI (protein disulfide isomerase) when treated with Z-VLL-CHO. The amyloid precursor protein (APP) and HS6ST3-GFP co-localize in the Golgi apparatus without Z-VLL-CHO treatment. However, they were found in the endoplasmic reticulum with Z-VLL-CHO treatment. The physiological significance of the co-localization of HS6ST3-GFP and APP is now under investigation.

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GPI anchor deficiencies: acquired and inherited

Taro Kinoshita (Osaka University)

More than 100 different proteins are inserted into the cell surface membrane via

GPI-anchor. Two GPI-anchor deficiencies, acquired and inherited, are known. Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by clonal blood cells having GPI-anchor deficiency caused by somatic mutation of PIGA, an X-linked gene required for the initial step of GPI biosynthesis. Several lines of evidence indicate that two mechanisms are involved in expansion of PNH clone: clonal selection mediated by autoimmunity to hematopoietic stem cells and benign tumor-like clonal expansion. We recently reported two patients whose PNH cells had chromosome 12 abnormalities, which caused ectopic expression of aberrant HMGA2 (implicated in mesenchymal benign tumors) in bone marrow.

Three patients with inherited GPI deficiency have recently been identified in two families. They had partial deficiency of GPI-anchored proteins on blood cells and fibroblasts, and had seizure and portal vein thrombosis. The same base substitutions were found within promoter region of PIGM, encoding mannosyltransferase for the first mannose. The mutation disrupted an SPI1-binding site, thereby, decreased histone H4 acetylation, resulting in low transcription of PIGM. The histone deacetylase inhibitor butyrate increased PIGM transcription and surface expression of GPI-anchored proteins *in vitro* and *in vivo*, ameliorating intractable seizure of one patient.

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Closing Remark