The 10th Symposium
of Japan Consortium for Glycobiology and Glycotechnology

Integration of Glycoscience and Beyond

November 29-30, 2012　at Tokyo Conference Center (Shinagawa)

Program November 29 (Thursday), 2012

Opening Address
Yukishige Ito (Organizing Chair, RIKEN)

Greetings from organizer
Toshisuke Kawasaki (JCGG President, Ritsumeikan University)

Special Lecture
Chair : Hisashi Narimatsu (AIST)

Unlock the secret of life with chemical compounds
Hiroshi Handa (Tokyo Institute of Technology)

Chemical compounds including pharmaceutical agents selectively bind to target proteins in the body and demonstrate their own effects by altering the function and structure of the targets. Accordingly, identification of targets is absolutely essential for understanding the mechanism of action of the drug and the network related the target. We have developed a novel magnetic-bead technology which enables one-step isolation/identification of a target protein from various protein libraries. Using the bead technology, we have identified target proteins for many drugs in the marketplace, such as tacrolimus (FK506), methotrexate, amino- bisphosphonate (alendronate), aspirin, lenalidomide, vesnarinone, ursodeoxycholic acid, and so on. Recently, we have successfully identified a causal factor of thalidomide teratogenicity. When DNA-binding transcription factors were isolated directly from nuclear extracts using DNA affinity beads, a protein kinase, sensitive to an adenosine analog DRB (5,6-dichloro-1-β-ribofuranosylbenzimidazole), was co-isolated. We focused on the point that DRB has an activity of inhibiting transcription elongation. Using the reconstituted system of a cell-free transcription assay, we discovered two novel factors, DSIF and NELF, involved in the temporal pausing of PolII at the transcription elongation step. We also found that this temporal pausing was released when PolII was phosphorylated by the protein kinase p-TEFb, sensitive to DRB, such that mRNA synthesis was resumed. We, therefore, elucidated the

mechanism of action of DRB. Further, it recently became apparent that a large part of genes are regulated by the temporal pausing, and that this regulation of PolII elongation is involved in neural differentiation, development, gene expression in response to external stimuli, control of pathogenic virus proliferation, epigenetic regulation, mRNA processing regulation, cell transformation, and so on.

References
1) Sakamoto S et al: Chem Rec 9, 66-85, 2009
2) Wada T et al.: Genes Dev 12, 343-56, 1998
3) Yamaguchi Y et al.: Cell 97, 41-51, 1999
4) Wada T et al.: EMBO J 17, 7395-403, 1998
5) Yamada T et al.: Mo! Cell 21, 227-37, 2006
6) Guo S et al.: Nature 408, 366-9, 2001
7) Yamaguchi Y et al.: Science 293, 124-7, 2001
8) Chen Y et al.: Genes Dev 23, 2765-77, 2009
9) Shimizu N et al.: Nat Biotechnol 18, 877-81, 2000
10) Ito T et al.: Science 327, 1345-50, 2010

Special Lecture

Chair : Akemi Suzuki (Tokai University)

Mechanism and regulation of ATP synthase

Masasuke Yoshida (Kyoto Sangyo University)

ATP synthesis is certainly the most prevalent chemical reaction in the biological world and the enzyme, ATP synthase, responsible for most of this task, is one of the most ubiquitous, abundant proteins on the earth. ATP synthase uses physical rotation of its own subunits as a step of catalysis. The driving force that spins ATP synthase is trans-membrane gradient of H^+ concentration that is generated by respiration or by sunshine. ATP synthase is bi-particle structure with a common rotary shaft. The flow of H^+ through the lower particle (F_0) drives the rotation of the central rotor that then forces upper particle (F_1) to make the bending motion for synthesis of ATP. In a reverse reaction, ATP hydrolysis drives rotation of F_1 that induces reverse protonpumping. Isolated F_1 by itself is an ATP-driven motor and how each elementary step of catalysis drives stepwise rotation of the central rotor subunit is well understood. ATP synthase would start wasteful ATP hydrolysis when cells are deficient of respiration, like ischemia. To prevent this, cells must have special regulation system, which is now being unveiled.

Program November 30 (Friday), 2012

Session 1 Functions and Fate of Glycan Chains

Chair : Tamao Endo (Tokyo Metropolitan Institute of Gerontology)

Synthesis based multiplexed approaches in glycoscience

Yukishige Ito (RIKEN)

Glycoproteins are characterized by their complexity and diversity. Development of synthetic methodologies useful for efficient and facile preparation of oligosaccharides is a focal issue in carbohydrate chemistry. In light of their structural diversity, practical strategy to facilitate the synthesis of oligosaccharide is expected to be highly valuable. Recent studies have clarified that protein glycosylation is not limit ed to eukaryotes, suggesting its widespread occurrence. In fact, various bacteria carry glycoproteins which are known to play crucial roles in the establishment of infection.

This talk will cover our recent results of 1) studies toward development of synthesis methods for eukaryotic and bacterial glycans, 2) analysis of glycoprotein functions using synthetic glycans and 3) glycoproteins, and mechanistic studies on D-mannose specific carbohydrate binding natural product. In addition, progress of the ERATO Glycotrilogy Project will be reported and its future perspective will be discussed.

Analyses of function of N-linked glycans in the ER using with a high mannose-type glycan library

Akira Seko (JST)

In the ER, high mannose-type N-linked glycans on glycoproteins are recognized by various quality control (QC)-associated proteins, in accordance with their folding states. However, for many QC associated proteins, precise molecular mechanism of their interactions with glycans remains unclear. We seek to clarify this issue using the approach based on synthetic chemistry. First, we developed a unique method for systematic preparation of a high mannose-type glycan library. This method is based on chemical synthesis of a tetradecasaccharide precursor that consists of Man9-oligosaccharide and capping sugar residues at the three nonreducing termini. Thirty-one high mannose-type glycans have been successfully prepared by sequential glycosidase digestions of the precursor.

Our attempt has also been directed to the synthesis of artificial glycoproteins. As a model, we employed dihydrofolate reductase (DHFR), which can bind to methotrexate (MTX) with high affinity. Several surface-located amino acid residues were substituted for Cys and then hydrophobic pylene residues were conjugated on the thiol groups. Man9-MTX was chemically synthesized and combined to pylene-

modified DHFR. We expect these molecules mimic glycoproteins having non-native conformations, which have surface exposed hydrophobic patches.

Now we have obtained various tools for analyses of biochemical roles of high mannose-type glycans on the QC system in the ER, and will go forward to elucidation of the QC system in a molecular level.

References

1) Matsuo I, Totani K, Tatami A, Ito Y: Comprehensive synthesis of ER related high-mannose-type sugar chains by convergent strategy. Tetrahedron, 62, 62-8277, 2006

2) Matsuo I, Wada M, Manabe S, Yamaguchi Y, Ohtake K, Kato K, Ito Y: Synthesis of monoglucosylated high- mannose-type dodecasaccharide, a putative ligand for molecular chaperone, calnexin, and calreticulin. J Am Chem Soc 125, 3402-3, 2003

Elucidation of the recognition mechanism of folding sensor enzyme UGGT by chemically synthesized intentionally misfolded glycoprotein

Masayuki Izumi, Yasuhiro Kajihara (Osaka University)

In the endoplasmic reticulum, a quality control (QC) system of glycoprotein functions to distinguish misfolded malfunctional glycoproteins from correctly folded one. In the QC system, UDP-glucose:glycoprotein glucosyltransferase (UGGT) is known as a folding sensor enzyme, which transfers glucose preferentially to misfolded glycoprotein bearing high-mannose type N-glycan which serves as a tag for refolding. To study glycoprotein QC system at molecular level, misfolded homogeneous glycoproteins bearing high-mannose type oligosaccharide were synthesized systematically as substrates of UGGT.

We designed a model glycoprotein based on interleukin 8 (IL-8), which is consisted of 72 amino acid residues and two disulfide bonds. Since IL-8 is not naturally glycosylated, we designed to incorporate high-mannose type (Man9 GlcNAc2) oligosaccharide to Asn36. Chemical synthesis of the full-length glycosylpolypeptide chain was accomplished by means of solid phase peptide synthesis and native chemical ligation. Extensive folding experiments of IL-8 polypeptide bearing Man9 GlcNAc2 oligosaccharide yielded correctly folded glycoprotein as well as two misfolded glycoproteins with non-native disulfide bond patterns and a disulfide bond-linked misfolded homodimer. Other glycoprotein models with one and no disulfide bond were also prepared. These synthetic homogeneous glycoprotein analogues were tested as substrates of UGGT and most of them were glucosylated but with different preferences. Among them, the most favored substrate was a homodimer which exhibits molten globule-like hydrophobic nature, and the least favored substrate was a correctly folded glycosyl-IL-8.

References
1) Izumi M, Makimura Y, Dedola S, Seko A, Kanamori A, Sakono M, Ito Y, Kajihara Y: J Am Chem Soc 134, 7238-41, 2012

Challenge to cellular glycan synthesis based on organic and analytical chemistry

Osamu Kanie (Tokai University)

A variety of molecules carrying glycan structures are secreted to extracellular space or the membrane surface where these molecules function. However, little is known about the mechanism of glycan synthesis. We focus on the glycan synthesis-related unsolved problems occurring at the endoplasmic reticulum and the Golgi apparatus. To investigate glycan structures of ER-resident glycoproteins, some of which are known to be N-glycosylated, we analyzed glycoproteins of ER extracts, and found that they carried short glycans. Further, synthetic fluorescently tagged molecules such as analogues of CMP-sialic acid and lactosyl ceramide are being used in investigating the process of glycosylation at Golgi apparatus. We also aim to develop a new method in atomic-force microscopy with enhanced resolution. A dendrimer with cyclodextrin as a core was synthesized. A reaction of the molecule at the tip of a cantilever would allow us to manipulate attaching single chemical probe molecule. This would then enhance both special and chemical resolutions, and would enable direct observation of a glycoprotein molecule.

References
1) Kanie O. Kanie Y, Daikoku S, Shioiri Y, Kurimoto A, Mutsuga S, Goto S, Ito Y, Suzuki K: Multistage mass spectrometric information obtained by deconvolution of energy-resolved spectra acquired by triple-quadrupole mass spectrometry. Rapid Commun Mass Spectrom 25, 1617-24, 2011
2) Suzuki K, Ohtake A, Ito Y, Kanie O: Synthesis of a fluorescently tagged sialic acid analogue useful for live-cell imaging. Chem Commun 48, 9744-46, 2012
3) Daikoku S, Ono Y, Ohtake A, Hasegawa Y, Fukusaki E, Suzuki K, Ito Y, Goto S, Kanie O: Fluorescence-monitored zero dead-volume nano-LC-micro ESI-QIT-TOF MS for analysis of fluorescently tagged glycosphingolipids. Analyst 136, 1046-50, 2011

Exploring the “life” of glycans

Tadashi Suzuki (RIKEN)

Cell surface glycans can be regarded as a "face" of cells, and their structures are known to change depending on developmental stages or environment. Therefore, cell surface glycans are utilized for identification of stem cells such as iPS/ES cells,

or as valuable biomarkers in diagnosis/detection of cancer. To understand the molecular mechanism of "change of face", biosynthetic pathway for glycan chains on glyco- proteins/glycolipids has been, in most part, revealed, while there are still many unresolved issues on their transport/degradation aspect. In this symposium, current knowledge for catabolic pathway of glycans in S. cerevisiae will be presented; we will be very surprised to realize that how little, in this so-called "post-genome" era, we know about such basic biological process. I will also present our new approach to visualize "glycoforms" of proteins of interest using trans- membrane FRET (fluorescence resonance energy transfer) technique. This technique will help us understand how glycan structures can affect the stability and/or intra/intercellular trafficking of carrier proteins.

References
1) Hirayama H et al.: J Biol Chem 285, 12390, 2010
2) Haga Y et al.: Nat Commun 3, 907, 2 012

JCGG Luncheon Seminar (sponsored by Shimazu, Co.)

Chair : Naoyuki Taniguchi (RIKEN)

Energy Resolved Oxonium Ion Monitoring (Erexim) technology for the analysis of N-glycan microheterogeneity on therapeutic antibodies

Koji Ueda (RIKEN)

The FDA draft guidance for the evaluation of biosimilarity was published in Feb 2012, in which emphasis was placed on the importance of evaluating minor structural differences that can significantly affect the potency and safety of biopharmaceuticals, with specific reference to glycosylation patterns on antibody drugs.

We here propose a new method for validating the micro- heterogeneity of N-glycans on therapeutic antibodies. The continuous collision energy scanning of oligosaccharide- derived fragment ions (oxonium ions) illustrated both comprehensive structural information of glycans on glycopeptides and their quantitative abundance simultaneously in a single analysis of a triple quadrupole mass spectrometer. The quantitation limit of glycopeptide was 30 attomole and, importantly, even very rare structural isomers were quantifiable, which existed only 0.1% of all glycoforms.

This technology, Energy Resolved Oxonium Ion Monitoring (Erexim), was applied to the evaluation of lot-to-lot glycoform variations of trastuzumab and bevacizumab. The re- suits showed gradually shortened tendency of the N-glycans on both drugs with time. We further utilized Erexim technology to in-depth analysis of cetuximab Fab glycosylation, previously known to cause anaphylaxis, and

quantitatively determined causative potential antigens including Lewis x motifs. We demonstrated herein an analysis scheme for routine evaluation of therapeutic biopharmaceuticals.

References
1) Toyama A, Nakagawa H, Matsuda K, Sato TA, Nakamura Y, Ueda K: Quantitative structural characterization of local N-glycan microheterogeneity in therapeutic antibodies by energy-resolved oxonium ion monitoring. Anal Chem 2012, in press.
※特願 2012-19790 8

Session 2　Mastering Chemical Reactions and Investing Glycan Chains

Chair : Osamu Kanie (Tokai University)

β-Glycosylation using 3,6-O-(o-xylylene)-bridged axial-rich glycosyl fluoride

Hidetoshi Yamada (Kwansei Gakuin University)

A glycosylation reaction using 3,6-0-(o-xylylene)- bridged axial-rich glucosyl fluoride demonstrated complete b-selectivity. The term 'axial-rich' refers to a conformation with more substituents in an axial than in an equatorial position of carbohydrates including the 1C4 and twist-boat forms. In this study, the o-xylylene-bridge between the 3- and 6-oxygens of D-glucopyranose locks the conformation to the axial-rich form.

The glucosyl fluoride efficiently reacted with various alcohols in a $SnCl_2$-$AgB(C_6F_5)_4$ catalytic system in benzotrifluoride with various alcohols including primary, secondary, tertiary, and sterically hindered hydroxy groups on sugars in good to excellent yields. The perfect β-stereoselectivity was observed in all cases; the α-isomers were not detected by ^{1}H NMR analysis of the crude products.

The mechanism composed of the glycosylation and isomerization cycles was revealed through comparative experiments using acidic and basic molecular sieves. The glycosylation cycle is triggered by the generation of $SnB(C_6F_5)_4Cl$ as the actual catalyst. This glycosylation is accompanied by the formation of HB(C $C_6F_5)_4$. The achieved perfect stereocontrol is attributed to the synergy of the axial-rich con-formation and convergent isomerization caused by HB(C $C_6F_5)_4$.

References
1) Okada Y, Asakura N, Sando M, Ashikaga Y, Yamada H: J Am Chem Soc 134, 6940-3, 2012
2) Yamada H: Trends Glycosci Glycotechnol 123, 122-33, 2011
3) Okada Y, Mukae T, Okajima K, Taira M, Fujita M, Yamada H: Org Lett 9, 1573-6, 2007

Challenges in glycosylation: Viewpoints of organic chemistry
Toshiki Nokami (Kyoto University)

Automated synthesis of oligosaccharides is one of the challenges of synthetic organic chemistry. Our approach is based on our finding that the electrochemical method is useful for conversion of thioglycosides to glycosyl triflates. These electrochemically generated glycosyl triflates are reactive enough to give the glycosylation products with various types of glycosyl acceptors including thioglycosides. Therefore, we are developing a novel automated synthesizer of -oligosaccharide based on the electrochemical method. Glycosyl cation is believed to be a highly reactive glycosylation intermediate with very short lifetime. Therefore, it is challenging to generate and accumulate glycosyl cations in conventional batch reactors even at very low temperature. Therefore, flow-micro reactor system which enables us to control the reaction time within 1.0 sec was used for activation of thioglycosides and the subsequent glycosylation via glycosyl cation or its equivalent. The activation of thioglycosides with an electrochemically generated activator ArS[ArSSAr]$^+$ gave the best result with a short reaction time (0.17 sec) at -28 ^{o}C. The details of this glycosylation using a flow micro reactor system will be presented.

References

1) Nokami T, Shibuya A, Tsuyama H, Bowers AA, Crich D, Suga S, Yoshida J: J Am Chem Soc 128, 10922-8, 2007

2) Nokami T; Nozaki Y; Saigusa Y, Shibuya A, Manabe S, Ito Y, Yoshida J: Org Lett 13, 1544-7, 2011

3) Saito K, Ueoka K, Matsumoto K, Suga S, Nokami T, Yoshida J: Angew Chem Int Ed 50, 5153-6, 2011

Synthesis and biological evaluation of β-glucan oligosaccharides
Hiroshi Tanaka (Tokyo Institute of Technology)

In this report, we describe the synthesis and biological evaluation of β(1,3) oligosaccharides that contain an aminoalkyl group and their biological evaluation. A 2,3 diol glycoside with a 4,6 benzylidene protecting group was used as an effective glycosyl acceptor for the synthesis of some (1,3) linked glycosides. The use of a combination of a linear tetrasaccharide and a branched pentasaccharide as glycosyl donors led to the preparation of β(1,3) linear octa- to hexadecasaccharides and branched nona- to heptadeca- saccharides in good total yields. Measurements of the competitive effects of the oligosaccharides on the binding of a soluble form of Dectin-1 to a solid-supported Schizophyllan (SPG) revealed that the branched heptadecasaccharide and the linear hexadecasaccharides also have binding activity for Dectin-1. In addition, the two oligosaccharides, both of which contain a (1,3) hexadecasaccharide backbone, exhibited agonist activity in a luciferase-assisted

NF-KB assay. STD-NMR analyses of complexes of Dectin-1 and the linear hexadecasaccharides clearly indicated Dectin-1 specifically recognized the sugar part of the oligosaccharides and not the aminoalkyl chain.

References
1) Brown GD, Gordon S: Nature 413, 36, 2001
2) Tanaka H, Kawai T, Adachi Y, Ohno N, Takahashi T: Chem Commun 46, 8249-51, 2010
3) Tanaka H, Kawai T, Adachi Y, Hanashima S, Yamaguchi Y, Ohno N, Takahashi T: Bioorg Med Chem 20, 3898- 914, 2012

Ganglioside synthesis oriented to the elucidation of functional mesoscopic domains in the cell membrane

Hiromune Ando (Gifu University)

Ganglioside, which is a family of sialic acid-containing glycolipids, is considered to have significant roles in the process of cell-cell signaling mediated by mesoscopic (1-100 nm) functional domains in the plasma membrane (=lipid rafts). Lipid raft is considered to be transiently assembled upon stimuli such as ligand biding to protein receptor, and gangliosides are known as key components of lipid raft. However, there is little known about how gangliosides participate in the assembly of the lipid raft and how they function in the lipid raft during signal transduction.

In this study, fluorescent probes of GM3 and GM1 that behave much like parent gangliosides in the cell membrane have been successfully synthesized for the first time. Next, the fluorescent probes were applied to single molecule imaging utilizing living cell. As a result, it was clearly demonstrated that ganglioside specifically colocalized with a raft domain of CD59 (GPI-anchored protein) in the cell membrane. Furthermore, a distinct difference in the affinity to EGF receptor between GM3 and GM1 was revealed by the single molecule observation.

References
1) Suzuki KGN, Kasai R S, Hirosawa KM, Nemoto YL, Ishibashi M, Miwa Y, Fujiwara TK, Kusumi A: Nat Chem Biol 8, 774-83, 2012
2) Ando H et al.: Tetrahedron Lett 44, 6883-86, 2003
3) a) Imamura A et al.: J Org Chem 74, 3009-23 (2009); b) Fujikawa K et al.: Chem Eur J 17, 5641-51, 2011

Session 3　Glycomes and Their Functions

Chair : Yukishige Ito (RIKEN)

Conformational flexibility of N-glycans in solution and its role on molecular recognition

Yuji Sugita, Wataru Nishima, Suyong Re (RIKEN)

Conformational diversity of glycans is essential for the specific binding to their receptor proteins. Each of multiple conformers of a glycan could serve a "key" for specific binding to a target protein. However, the labile nature of glycans makes characterizing their conformational states a challenging issue. Here, we performed replica-exchange molecular dynamics (REMD) simulations to identify a family of multiple conformers of N-glycans in solution. The results provide new insights into the conformational equilibria of N-glycans and their alternation by chemical modification, supporting the concept of "conformer selection" in protein-glycan recognition. We also emphasize the importance of statistical averaging over the multiple conformers of glycans for comparing simulation results with experimental observables. Further theoretical developments prompt to explore the relationship between the flexibility of glycans and their specific recognition.

References

1) Re S, Miyashita N, Nishima W, Sugita Y: Conformational flexibility of N-glycan in solution revealed by REMD simulations. Biophys Rev 4, 179-87, 2012

2) Nishima W, Miyashita N, Yamaguchi Y , Sugita Y, Re S: Effect of bisecting GlcNAc and core fucosylation on conformational properties of biantennary complex-type N-glycans in solution. J Phys Chem B 116 (2012) 8505-12, 2012

3) Re S, Miyashita N, Yamaguchi Y, Sugita Y: Structural diversity and changes in conformational equilibria of biantennary complex-type N- glycans in water revealed by replica-exchange molecular dynamics simulation. Biophys J (Let), 101, L44-6, 2011

Systems chemical biology: Integration of bioinformatics and chemo-informatics

Yasushi Okuno (Kyoto University)

By the end of the 1960s, computing had reached a stage mature enough to be applicable to biochemical problems of limited scope, and the first generation of bioinformatics and chemoinformatics was born. Continuing into the next decade, evolutionary trees were one bioinformatics topic, and chemoinformatics topics such as the efficient representation of chemicals for searchable databases were explored. Computing technology was slowly becoming a useful tool to explore the theoretical underpinnings of the information representing the mechanisms of life.

Both bioinformatics and chemoinformatics have emerged independently in parallel, much like computing and molecular biology did at first. Their synergy was largely ignored, not for lack of interest, but rather because the computing power necessary to examine and solve large chemical biology problems that impact drug design was still insufficient. In this presentation, we focus on our recent study of "Systems chemical biology", that represents efforts to unify the bioinformatics and chemoinformatics aspects. Systems chemical biology is needed for comprehensive analysis of multiple interactions between proteins and compounds and for computational molecular design of drugs.

Crystal structures of 1,3-1,4-α-L-fucosidase that specifically recognizes Lewis a/x antigens

Shinya Fushinobu (University of Tokyo)

Lewis a [Galβ1-3(Fucα1-4)GlcNAc-; Le^a] and Lewis x [Galβ1-4(Fucα1-3)GlcNAc-; Le^x] blood group epitopes play fundamental roles in various biological processes such as mammalian cell-to-cell communications and host-pathogen interactions. 1,3-1,4-α-L-Fucosidases belonging to the subfamily B of Glycoside Hydrolase family 29 (GH29-B) have strict substrate specificities to $Le^{a/x}$ epitopes. We have determined the crystal structures of a GH29-B 1,3-1,4-α-L-fucosidase from *Bifidobacterium longum* subsp. *infantis* complexed with deoxyfuconojirimycin and lacto-N-fucopentaose II, providing the structural basis for its unique substrate specificity. A fucosynthase that specifically introduces $Le^{a/x}$ antigens into type-1/2 chains (lacto-N-biose I and N-acetyllactosamine) was also generated from a bifidobacterial GH29-B 1,3-1,4-α-L-fucosidase by the glycosynthase technique.

References

1) Ashida H, Miyake A, Kiyohara M, Wada J, Yoshida E, Kumagai H, Katayama T, Yamamoto K: Glycobiology 19, 1010-7, 2009.

2) Sakurama H, Fushinobu S, Hidaka M, Yoshida E, Honda Y, Ashida H, Kitaoka M, Kumagai H, Yamamoto K, Katayama T: J Biol Chem 287, 16709-19, 2012

Cell growth modulation through C-mannosylated peptides

Yoshito Ihara (Wakayama Medical University)

C-Mannosylation occurs at the first Trp in the Trp-X-X-Trp motif, which is found in the thrombospondin type 1 repeat (TSR) superfamily proteins, such as thrombospondin, properdin, and F-spondin. To investigate whether C-mannosylation in the TSR plays functional roles in the cell, we examined the effect of synthesized C-

mannosylated TSR-derived peptides on TGF-P signaling in cultured fibroblast cells. We found that TGF-P-induced cell proliferation was suppressed by the C-mannosylated peptides, compared to the peptides without mannose. In the presence of the C-mannosylated peptides, TGF-P-induced phosphorylation and nuclear accumulation of Smad2 were suppressed, resulting in decrease in the expression of collagen type I. These results suggest that C-mannosylated TSR plays a functional role in the regulation of TGF-P signaling. We also searched specific binding partners for C-mannosylated peptides in the cells, and isolated several candidate proteins. Peptide mass finger printing followed by a data base search suggested that the proteins are Hsc70, myosin-1c, and vimentin. In this symposium, we would like to demonstrate our recent results concerning the functional relevance of the binding proteins to C-mannosylated peptides in the TGF-β-induced cell signaling.

References

1) Ihara Y, Inai Y, Ikezaki M: Protein C-mannosylation and its prospective functions in the cell. Trends Glycosci Glycotechnol 23, 1-13, 2011

2) Manabe S, Ito Y: Total synthesis of novel subclass of glyco-amino acid structure motif: C2-α-C-mannosylpyranosyl-L-tryptophan. J Am Chem Soc 121, 9754-5, 1999

3) Ihara Y, Manabe S, Ikezaki M, Inai Y, Matsui I-SL, Ohta Y, Murai E, Ito Y: C-Mannosylated peptides derived from the thrombospondin type 1 repeat interact with Hsc70 to modulate its signaling in RAW264.7 cells. Glycobiology 20, 1298-310, 2010

Glycobiology in the field of aging research

Tamao Endo (Tokyo Metropolitan Institute of Gerontology

Glycoproteins work as a biosignal for multi-cellular organisms, including cell-cell adhesion, cell-matrix adhesion, and extracellular receptor-ligand interactions. Therefore, it is important to analyze the changes of glycoproteins in aging. We found the accumulation of fourteen N-glycosylated proteins in the rat cerebral cortex cytosolic fraction in the aging process by a comparative study with two-dimensional gel electrophoresis and concanavalin A staining. All proteins had high mannose N-glycans because they were sensitive to endoglycosidase H digestion. Then, three cytosolic glycoproteins were identified as cathepsin D. The increase of cytosolic cathepsin D during aging was not due to lysosomal membrane disruption because other lysosomal enzymes did not increase in the cytosolic fraction. Taken together, we propose that cytosolic cathepsin D is a new biomarker of aging. Later, we found that oxidative stress and the suppression of proteasome activity triggered the translocation of cathepsin D from lysosomes to cytosol. Additionally, we performed proteomic analysis of plasma proteins in Japanese semisuper centenarians (SSCs)

(>105 years) and young controls (20-39 years), and found that 18 protein spots were altered in the plasma of SSCs. Proteins related to oxidative stress were altered in SSCs, suggesting that systemic redox regulation is important for the longevity of SSCs.

References
1) Sato Y, Suzuki Y, Ito E, et al.: Mech Ageing Dev 127, 771-8, 2006
2) Miura Y, Sato Y, Arai Y, et al.: Exp Gerontol 46, 81-5, 2011

Closing Remarks