The 12th Symposium
of Japan Consortium for Glycobiology and Glycotechnology

Glycan-Based Therapeutics and Beyond
Infection, Inflammation, Immunity and Glycoscience

December 4-5, 2014 at Tokyo Medical and Dental University (Tokyo)

Program December 4 (Thursday), 2014

Greetings from JCGG President
Naoyuki Taniguchi (RIKEN)

Opening Address of Organizer
Takeshi Tsubata (Tokyo Medical and Dental University)

Session 1　Toward the Developing of Novel Adjuvants (1)
Chair : Tatusro Irimura (St. Luke's International Hospital)

Mechanism for immune activation induced by synthetic sialosides
Takeshi Tsubata (Tokyo Medical and Dental University)

CD22/Siglec-2 is a member of the Siglec family that specifically recognizes α2,6 sialic acid. CD22 contains immunoreceptor tyrosine-based inhibition motifs (ITIMs) at the cytoplasmic tail and negatively regulates B cell receptor (BCR) signaling. α2,6 sialic acid is abundantly expressed in B cells, and the ligand binding site of CD22 on B cells is mostly occupied by the ligand expressed on the same cell (cis ligand). How the interaction of CD22 with the cis ligand regulates CD22 is controversial. There are pieces of evidence suggesting that the cis ligand positively or negatively regulates CD22, respectively. We have developed synthetic sialosides that bind to CD22 with sub-micromolar affinity whereas α2,6 sialic acid binds to CD22 with millimolar affinities. Among these synthetic sialosides, GSC718 contains a biphenyl group at the C9 position and a benzyl group at the C2 position. In vitro treatment with GSC718 enhanced activation and proliferation of mouse spleen B cells probably by disrupting the interaction of CD22 with the cis ligand. Interestingly, GSC718 failed to enhance Ca^{+2} signaling induced by BCR ligation. These results suggest that the cis ligand of CD22 regulates B cell activation independently of BCR ligation-induced signaling.

Design and synthesis of the ligands as well as inhibitors to elucidate the biological functions of animal lectins

Hideharu Ishida (Gifu University)

A series of the ligands as well as the inhibitors of siglec2 expressed on B cells were designed and synthesized to elucidate the biological functions of siglec2. Improvements in the chemical synthesis of sialosides as well as the assay system using the synthetic probes facilitated the SAR study. A novel, small molecule inhibitor of siglec2 was developed as a promising candidate to enhance immune system.

References

1) Abdu-Allah HHM, Tamanaka T, Yu J, Zhuoyuan L, Sadagopan M, Adachi T, Gao X, Tsubata T, Kelm S, Ishida H, Kiso M: Design, synthesis, and structure-affinity relationships of novel series of sialosides as CD22-specific inhibitors. J Med Chem 51, 6665-81, 2008

2) Abdu-Allah HHM, Watanabe K, Hayashizaki K, Iwayama Y, Takematsu H, Kozutsumi Y, Tsubata T, Ishida H, Kiso M: Synthesis of biotinylated sialosides to probe CD22-ligand interactions. Tetrahedron Lett 50, 4488-91, 2009

3) Abdu-Allah HHM, Watanabe K, Completo GC, Magesh S, Hayashizaki K, Takaku C, Tamanaka T, Takematsu H, Kozutsumi Y, Paulson JC, Tsubata T, Ando H, Ishida H, Kiso M: CD22-Antagonists with nanomolar potency: The synergistic effect of hydrophobic groups at C-2 and C-9 of sialic acid scaffold. Bioorg Med Chem 19, 1966-71, 2011

Affinity and specificity on lectin-sugar interaction

Yoshiki Yamaguchi (RIKEN)

Lectins are widely recognized as valuable tools to detect the specific glycan structures. In addition, data are accumulating to show the essential roles of lectins in many biological processes. Recently, many 3-D structural data are available for the lectin-sugar complexes, explaining how the lectin specifically binds to the glycan. Based on these atomic information, "lectin-blocking drugs" will be developed to fight against infection, inflammation and tumor metastasis. Siglecs are a membrane-bound glycoprotein that specifically binds to sialic acid. The N-terminal V-set domain, which is responsible for the binding to sialic acid, is characterized by its intra-sheet disulfide bond and splitting of the G strand. The inter-sheet separation seems to expose two aromatic side chains, which play key roles in interacting with sialic acid. The unpaired G strand makes hydrogen bonds with sialic acid. We performed saturation transfer difference (STD)-NMR and transferred (tr) NOE analyses for the interaction between Siglec-2 (CD22) and its inhibitors. NMR data provides the information on the group epitope and lectin-bound conformation

of the inhibitors. The inhibitor-binding site on Siglec can be obtained by stable-isotope assisted NMR techniques. Development of lectin inhibitors with high affinity and high specificity will be discussed in terms of rational design.

References
1) Hanashima S, Sato K, Naito Y, Takematsu H, Kozutsumi Y, Ito Y, Yamaguchi Y: Synthesis and binding analysis of unique AG2 pentasaccharide to human Siglec-2 using NMR techniques. Bioorg Med Chem 18(11), 3720-5, 2010
2) Hanashima S, Sato C, Tanaka H, Takahashi T, Kitajima K, Yamaguchi Y: NMR study into the mechanism of recognition of the degree of polymerization by oligo/polysialic acid antibodies. Bioorg Med Chem 21(19), 6069-76, 2013
3) Nagae M, Morita-Matsumoto K, Kato M, Kaneko MK, Kato Y, Yamaguchi Y: A platform of C-type lectin-like receptor CLEC- 2 for binding 0-glycosylated podoplanin and non-glycosylated rhodocytin. Structure 2014, in press, http://dx.doi.org/10.1016/ j.str.2014.09.009

Special Lecture I
Chair : Takeshi Tsubata (Tokyo Medical and Dental University)

Adjuvant data base project: New mode of action and biomarker
Ken J Ishii (NIBIO)

The research and development of adjuvants has a history of more than 80 years, and their actual mechanism was not immunologically understood for a long time, with a famous sarcastic remark "Immunologist's dirty little secret". Recent advance in Immunolgy; however, allowed the development of adjuvants through an innovative scientific approach, and there is fierce competition world wide for the development of next-generation adjuvants.

On the other hand, however, adjuvants range widely in terms of origin and mode of action, and they may be the cause or underlying cause of vaccine toxicity, especially immunotoxicity.

To improve both efficacy and safety of vaccine adjuvant, we initiated the adjuvant database project 3 years ago. Animal and human experimental data based on tissue mRNA and miRNA array system are being collected. I would like to introduce several adjuvant with new mechanism of action and potential biomarkers based on this adjuvant data base analysis.

We expect this adjuvant database can provide useful information to understand the mechanism of a new adjuvant, and to predict the host responses to the adjuvants and the adjuvanted vaccines.

References

1) 著書 :「 アジュバント開　発研 究の 新展開 」石井健 、山西弘一編、CMC 出版 2011
2) Kuroda E, Coban C, Ishii KJ: Particulate adjuvant and innate immunity: past achievements, present findings, and future prospects. Int Rev Immunol 32(2), 209-20, 2013.
3) Jounai N et al.: Recognition of damage-associated molecular patterns related to nucleic acids during inflammation and vaccination. Front Cell Infect Microbiol 2, 168, 2012
4) Desmet CJ, Ishii KJ: Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination. Nat Rev Immunol 12(7):479-91, 2012

Session 2　Toward the Developing of Novel Adjuvants (2)
Chair : Takeshi Tsubata (Tokyo Medical and Dental University)

Immune responses against glycolipids through C-type lectin receptors
Akira Yamazaki (Kyushu University)

C-type lectin receptors (CLRs) comprise a family of proteins that share a common structural motif and are involved in various immune responses, and identified as pattern recognition receptors (PRRs) following TLRs, NLRs and RIRs. We found that Mincle (Macrophage-inducible C-type lectin) is an FcRy-coupled activating receptor that recognizes dead cells to induce inflammatory cytokines. We also found that Mincle also for a mycobacterial glycolipid, cord factor (also called trehalose dimycolate, TDM). TDM-mediated innate immunity and granuloma formation were completely dependent on Mincle. We recently identified that another ITAM-coupled CLR, MCL and Dectin-2, recognize mycobacterial glycolipids and mediate their adjuvanticity. These results suggest that these CLRs may act as sensors for damaged-self (DAMPs) and non-self pathogens (PAMPs) to activate innate and acquired immunity. The physiological advantage and potential risk of the recognition of glycolipids through CLRs will be discussed.

References
!) Yonekawa A, Saijo S, Hoshino Y, Miyake Y, Ishikawa E, Suzukawa M, Inoue H, Tanaka M, Yoneyama M, Oh-hora M, Akashi K, Yamasaki S: Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. Immunity 41, 402-13, 2014
2) Miyake Y, Toyonaga K, Mori D, Kakuta S, Hoshino Y, Oyamada A, Yamada H, Ono K, Suyama M, Iwakura Y, Yoshikai Y, Yamasaki S: C-Type lectin MCL is an FcRy-coupled receptor that mediates the adjuvanticity of mycobacterial cord factor. Immunity 38,1050- 62, 2013
3) Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi 0, Kinoshita T, Akira S, Yoshikai Y, Yamasaki S: Direct recognition of the mycobacterial glycolipid,

trehalose dimycolate, by C-type lectin Mincle. J Exp Med 206, 2879-88. 2009

Development of a new pneumococcal vaccine with glycolipid

Yuki Kinjo (NIID)

Pneumococcus is a major causative bacterium of pneumonia that is the third leading cause of death in Japan. The current pneumococcal polysaccharide-based vaccines have reduced the burden of invasive pneumococcal infection. However, the incidence rate of invasive infection with non-vaccine serotypes has been dramatically increasing. Therefore, it is important to develop new pneumococcal vaccines that are effective for non-vaccine serotypes. PspA, a protein present in all serotypes has been proposed as a promising candidate for a new vaccine. We have found that innate type lymphocytes called iNKT cells play an important role in the early defense against pneumococcal infection through the recognition of bacterial glycolipid. When iNKT cells are stimulated with glycolipids, they augment subsequent acquired immune responses through the maturation of dendritic cells. We hypothesized that glycolipid mediated iNKT cell activation would be useful for new vaccine development, and we tested the protective effect of immunization with glycolipid and PspA against pneumococcal infection. The immunized mice exhibited higher survival rate and lower bacterial burden in the lung than control mice. The plasma anti-PspA IgG titers were significantly higher in the immunized mice. These results suggest that glycolipid mediated iNKT cell activation is useful for development of new pneumococcal vaccines.

References

1) 河原隆二, 他：<速報> 2013 年度の侵襲性肺炎球菌感染症の患者発生動向と成人患者由来の原因菌の血清型分布―成人における血清型置換 (serotype replacement) について―. IASR 35, 179-181, 2014
2) Kinjo Y et al.: Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. Nat Immunol 12, 966-974, 2011
3) Miyasaka T et al.: A possible relationship of natural killer T cells with humoral immune response to 23-valent pneumococcal polysaccharide vaccine in clinical settings. Vaccine 30, 3304-10, 2012

Immunomodulatory glycoconjugates from microbes: Structure-activity relationships and the application

Yukari Fujimoto (Keio University)

Cell surface components from microbes often show immuno-modulatory activities. Our group have synthesized these compounds and applied them for the analysis of their structural recognition and the activation system of innate immunity.

The components include bacterial cell wall peptidoglycan (PGN; Nodl and Nod2 ligands), lipoproteins/lipopeptides (LP; TLR2 ligands), and also other glyco- or lipid-conjugates as the ligands of TLR4 or other lipid antigen receptors. We have investigated the immunomodulatory activities and the adjuvant activities of these microbial glyco- and lipid-conjugates with developing the chemical synthesis. For the development of the effective immune adjuvant, we have also studied a conjugated immune adjuvant structures.

References
1) Fujimoto Y, Pradipta AR, Inohara N, Fukase K: Nat Prod Rep 29, 568, 2012.
2) Fujimoto Y, Hashimoto M, Katsumoto M, Fukase K et al.: ChemBioChem 10, 2311, 2009
3) Azuma M, Sawahata R, Akao Y, Ebihara T, Yamazaki S, Matsumoto M, Hashimoto M, Fukase K, Fujimoto Y, Seya T: PLoS One 5, el 2550, 2010

Session 3　Inflammation and Its Control with Glycoscienc

Chair : Yuki Kinjo (NIID)

PILR regulate immune response via sugar chain recognition

Hisashi Arase (Osaka University)

PILRα is one of paired receptors that consist of inhibitory and activating receptors. PILRα recognizes CD99 as one of host ligands and herpes simplex virus glycoprotein B (gB) as a viral ligand. Analyses of interaction between PILR and these ligands revealed that sialic acid-containing O-glycan structure on the ligands are required for the interaction. Structural analyses of PILRα bound to gB-derived glycan peptide demonstrated that PILRα exhibit binding sites to both sugar chain and peptide. Furthermore, structure of PILRα was modified by association with the ligand, suggesting that structural change of PILRα plays an important role in membrane fusion during viral infection.

References
1) Satoh T, Arii J, Suenaga T, Wang J, Kogure A, Uehori J, Arase N, Shiratori I, Tanaka S, Kawaguchi Y, Spear PG, Lanier LL, Arase H: PILRα is a herpes simplex virus-1 entry co-receptor that associates with glycoprotein B. Cell 132, 935-44, 2008
2) Wang J, Shiratori I, Uehori J, Ikawa M, Arase H: Neutrophil infiltration during inflammation is regulated by PILRα via modulation of integrin activation. Nat Immunol 14, 34-40, 2013
3) Kuroki K, Wang J, Ose T, Yamaguchi M, Tabata S, Maita N, Nakamura S, Kajikawa M, Kogure A, Satoh T, Arase H, Maenaka K: Structural basis for simultaneous recognition of an O-glycan and its attached peptide of mucin family by immune

receptor PILRα. Proc Natl Acad Sci USA 111, 8877-82, 2014

Macrophage galactose-type C-type lectin (MGL/CD301) in inflammation and immunity

Kaori Denda (St. Luke's International University)

Involvement of the macrophage galactose-type C-type lectin (MGL/CD301) expressed on macrophages and dendritic cells (DCs) in the regulation of inflammation and immunity was investigated with KO-mice and other means. In mice, MGL1/2 expressing cells are observed in the connective tissues throughout the body but the function seems to be diverse as stated herein.

In skin and skin-draining LNs, a dermal DC subset expressing MGL1/2 ($MGL2^+$ dDCs) induces the Th2-type immune response *in vivo* in a model of contact hypersensitivity. By targeting $MGL2^+$ dDCs with a rat monoclonal antibody against MGL2, a Th2-type humoral immune response is efficiently induced. *Mgl1*-KO mice show significantly severer inflammation than wild-type mice after oral administration of dextran sulfate sodium salt (DSS) and commensal bacteria are shown to induce IL-10 expression in $MGL1^+$ colonic lamina propria macrophages, suggesting that MGL1 plays a suppressive role in the regulation of inflammation potentially induced by commensal bacteria at the bowel wall. MGL1 with its hemITAM motif in its cytoplasmic domain may participate in the signal transduction, which is likely to lead to IL-10 production in lamina propria macrophages.

References

1) Murakami R, Denda-Nagai K, Hashimoto S, Nagai S, Hattori M, Irimura T: A unique dermal dendritic cell subset that skews the immune response toward Th2. PLoS One 8:e73270, 2013

2) Denda-Nagai K, Aida S, Saba K, Suzuki K, Moriyama S, Oo-Puthinan S, Tsuiji M, Morikawa A, Kumamoto Y, Sugiura D, Kudo A, Akimoto Y, Kawakami H, Bovio NV, Irimura T: Distribution and function of macrophage galactose-type C-type lectin 2 (MGL2/ CD30 lb): Efficient uptake and presentation of glycosylated antigens by dendritic cells. J Biol Chem 285, I9193-204, 2010

3) Saba K, Denda-Nagai K, Irimura T: AC-type lectin MGL1/CD30la plays an anti-inflammatory role against murine experimental colitis. Am J Pathol 174, 144-52, 2009

The role of CDIR in maintaining immune and bone homeostasis

Yoichiro Iwakura (Tokyo University of Science)

C-type lectin receptors (CLRs) are a group of transmembrane proteins with one or more extracellular carbohydrate recognition domains in a calcium-dependent

manner. Dendritic cell immunoreceptor (*Clec4a2*, DCIR) is one of CLR family members and is predominantly expressed in dendritic cells (DCs) and macrophages. DCIR has an ITIM in the cytoplasmic region. We showed that aged *Dcir*$^{-/-}$ mice spontaneously develop autoimmune sialadenitis and enthesitis, and are highly susceptible to collagen-induced arthritis. This is because DCIR negatively regulates differentiation and proliferation of DCs by suppressing GM-CSF signaling; DC population is excessively expanded in *Dcir*$^{-/-}$ mice, causing development of autoimmunity and highly susceptible to antigen stimulation. Thus, DCIR is critically important for the homeostasis of the immune system.

Furthermore, we found that aged *Dcir*$^{-/-}$ mice spontaneously developed ankylotic arthropathy. We showed that articular changes were completely suppressed in *Rag2*$^{-/-}$ mice, indicating an immune-mediated pathology. Furthermore, joint ankylosis was completely abolished in *Tnf*$^{-/-}$*Dcir*$^{-1-}$ and *Ifng*$^{-/-}$ *Dcir*$^{-/-}$ mice, but not in *Il17a*$^{-/-}$*Dcir*$^{-/-}$ mice, suggesting that TNF and IFN-γ are crucial for the development of ankylosis. We also showed that IFN-γ-producing T cells are efficiently induced in *Dcir*$^{-/-}$ mice, and IFN-γ has potent chondrogenic and osteogenic activity. These observations suggest that DCIR and IFN-γ are possible targets for the treatment of bone metabolic diseases.

Industry-Academia Joint Seminar (sponsored by Tokyo Chemical Industry Co.)
Modification of Glycan Structures for Glycoprotein-Drug Discovery

Approach toward industrialization of glycans until today : GLIT activities and Glycobiologics research meeting
Jun Hirabayashi (AIST)

Addition and remodeling of glycans by use of microbial endoglycosidase
Kenji Yamamoto (Ishikawa Prefectural University)

Technological development of glycan addition by using Endo-M
Toshiyuki Inazu (Tokai University)

Artificial functional glycans and newly development of Neoglycobiologics by use of endoglycosidase
Koji Ito (Tokushima University)

Program December 5 (Friday), 2014

Special Lecture I
Chair : Naoyuki Taniguchi (RIKEN)

Treatment for chronic obstructive pulmonary disease: History, current strategies, and development of personalized medicine
Kozui Kida (Nippon Medical School)

More than 7 million Japanese adults suffer from chronic obstructive pulmonary disease (COPD), which hampers the daily work, limits exercise, and disturbs routine social achv1t1es.

The current understanding of COPD is based on research that dates back to the mid-1960s. Initial research was based on pathological studies, which were then followed by physiological approaches. In 1968, an airway hypothesis was proposed in a first pathophysiological study. This study introduced an entirely new perspective on COPD, which has significantly influenced our understanding of the disease over the past two decades. In 2001, release of the Global Strategy for the Diagnosis, Management, and Prevention of COPD (GOLD) document has further contributed to our understanding. In 2010, a group of experts proposed the concept of a clinical COPD phenotype. Personalized medicine, as known P4 medicine, has become a treatment concept for COPD. A multi-level (environmental, clinical, biological, and genetic) approach is required, which will enable development of novel treatment approaches with robust clinical application in the future.

Session 4　Molecular Pathology, Diagnosis and Therapy of COPD
Chair : Koichi Honke (Kochi University)

Pathophysiology and treatment of acute exacerbation of chronic obstructive pulmonary disease (COPD)
Tomoko Betsuyaku (Keio University)

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide and results in an economic and social burden that is both substantial and increasing. Exacerbations of COPD are very important in clinical course of COPD, as they adversely affect a patient's prognosis and quality of life. It is reported that the patients who have frequent exacerbations belong to a clinically distinct (particular) phenotype that is susceptible to further exacerbations. Several factors can identify populations at risk of exacerbations. Implementing prevention measures in patients at risk is a major goal in the management of COPD. However, the clinical characteristics or percentage of the exacerbations on each severity have not been clarified yet. Keio University and affiliated hospitals have established an

observational COPD cohort. We annually check individuals' pulmonary function and CT scans. Exacerbations and comorbidities were identified based on patients' reports and physicians' records. The patients who had exacerbations of any severity tend to have exacerbations of the same severity in the next year. The patients who had exacerbations, even mild ones, showed worse than exacerbation-free patients. Evidence-based guidelines stipulate that early detection and prompt treatment of exacerbations are essential to ensure optimal outcomes, however, the treatment strategy is not optimal. Future direction will be discussed.

Genetic predictors and biomarkers of COPD exacerbations

Tateo Ishii (Nippon Medical School)

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease characterized by irreversible airflow limitation induced mainly by smoking. COPD exacerbation, or episodic worsening of symptoms, often results in hospitalization and increased mortality. Airway infections by bacterial strains, such as nontypeable *Haemophilus influenzae* (NTHi), are major causes of COPD exacerbation. NTHi express lipooligosaccharides that contain sialic acids, and may interact with Siglec-14, a "lectin" expressed on myeloid cells, which recognizes sialic acids and serves as an activating signal transduction receptor. NTHi interacted with Siglec-14 to enhance proinflammatory cytokine production and loss of Siglec-14, due to *SIGLEC14*-null allele homozygosity, was associated with a reduced risk of COPD exacerbation (Figure 1)[1]. Since soluble mediators secreted by myeloid cells responding to Siglec-14 engagement could be involved in the pathogenesis of exacerbation, we sought genes induced by NTHi in Siglec-14^+ myeloid cells and evaluated their utility as biomarkers of COPD exacerbation. Serum concentration of interleukin-27 was elevated in COPD patient sera during exacerbation[2]. Lectins involved in host defense such as Siglec-14, that could also trigger exaggerated response, might generate unwanted local and systemic inflammation, which could be detrimental to a host and could generate COPD with a frequent-exacerbation phenotype, its progression, and its comorbidities (Figure 2)[3].

References

I) Angata T, Ishii T, Motegi T, Oka R, Taylor RE, Soto PC, Chang YC, Secundino I, Gao CX, Ohtsubo K, Kitazume S, Nizet V, Varki A, Gemma A, Kida K, Taniguchi N : Loss of Siglec-14 reduces the risk of chronic obstructive pulmonary disease exacerbation. Cell Mol Life Sci 70 (17), 3199-210, 2013

2) Angata T, Ishii T, Gao C, Ohtsubo K, Kitazume S, Gemma A, Kida K, Taniguchi N: Association of serum interleukin-27 with the exacerbation of chronic obstructive pulmonary disease. Physiol Rep 2 (7) el 2069, 2014

3) Ishii T, Kida K: Predictors of chronic obstructive pulmonary disease exacerbations.

Curr Opin Pulm Med 20 (2), 138-45, 2014

Effect of glycosaminoglycan to cigarette smoke-induced emphysema model mice

Manabu Ueno (Takasaki General Medical Center)

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease caused by inhaling toxic gases represented by cigarette smoke. COPD is a complex inflammatory disease that involves many inflammatory cells, such as neutrophils, macrophages and $CD8^+$ T cells. These cells produce a lot of inflammatory mediators including cytokines, chemokines, and proteases, which induce destruction of lung parenchyma and airflow limitation.

αl,6-Fucosyltransferase (Fut8) is altered under pathological conditions. A deficiency in core fucosylation caused by the genetic disruption of Fut8 leads to the development of emphysematous lesions by attenuation of TGF-β1 receptor signaling. Moreover, $Fut8^{+/-}$ mice developed cigarette smoke-induced emphysema associated with an activation of MMPs (matrix metalloproteinases).

We develop new therapy using glycosaminoglycan for suppression of chronic inflammation and interruption of pathological progression in COPD. First, we established exacerbation model by single intratracheal administration of LPS to mice with cigarette smoke-induced emphysema provoked infiltration of inflammatory cells, MMPs production and air space enlargement.

Next, we treated with Keratan sulfate (L4) intratracheally after LPS administration to $Fut8^{+/-}$ exacerbation model mice. L4 instillation inhibited an increase of inflammatory cells in alveolar spaces and suppressed production of MMPs. The present drug would provide the beneficial effect on COPD.

References

1) Wang X, Inoue S, Gu J, Miyoshi E, Noda K, Li W, Mizuno-Horikawa Y, Nakano M, Asahi M, Takahashi M, Uozumi N, !hara S, Ho Lee S, Ikeda K, Kondo A, Shapiro SD, Lopez-Olin C, Kuwaki T, Okabe M, Honke K, Taniguchi N: Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. Proc Natl Acad Sci USA 102, 15791-6, 2005

2) Gao C, Maeno T, Ota F, Ueno M, Korekane H, Takamatsu S, Shirato K, Matsumoto A, Kobayashi S, Yoshida K, Kitazume S, Ohtsubo K, Betsuyaku T, Taniguchi N: Sensitivity of heterozygous α1,6-fucosyltransferase knock-out mice to cigarette smoke-induced emphysema: implication of aberrant transforming growth factor-β signaling and matrix metalloproteinase gene expression. J Biol Chem 287, 16699-708, 2012

3) Kobayashi S, Fujinawa R, Ota F, Kobayashi S, Angata T, Ueno M, Maeno T,

Kitazume S, Yoshida K, Ishii T, Gao C, Ohtsubo K, Yamaguchi Y, Betsuyaku T, Kida K, Taniguchi N: A single dose of lipopolysaccharide into mice with emphysema mimics human chronic obstructive pulmonary disease exacerbation as assessed by micro-computed tomography. Am J Respir Cell Mol Biol 49, 971-7, 2013

The role of oligosaccharides on the progress of chronic obstructive pulmonary disease

Congxiao Gao (RIKEN)

Chronic obstructive pulmonary disease (COPD) is a severe, slowly progressive and disabling disease associated with an accelerated decline in lung function. Emphysema and chronic bronchitis are its important phenotypes. Currently, the available therapies for COPD are relatively ineffective, as there are no drugs available that are capable of reducing the progression of the disease or mortality or the incidence of exacerbations. In order to well understand the role of oligosaccharides in COPD, we have established αl, 6-fucosyltransferase (Fut8) heterozygous knockout mice for cigarette smoke (CS) exposure. CS decreased the enzyme activity and elevated the matrix metalloproteinase(MMP)-9 activities, which led to the early-onset of emphysema. Furthermore, when we focus on the glycosaminoglycan in lung, we found that KS disaccharide could specifically block the interaction of flagellin with TLRS and subsequently suppress IL-8 production in normal human bronchial epithelial cells. While this oligosaccharide is administered to elastase-induced emphysema model mice, we found significantly reduced neutrophil influx and, MMP activity in bronchial alveolar (BAL), and the alveolar destruction was decreased. In this symposium, we would like to introduce our recent studies about glycoscience research on COPD.

References

I) Gao C, Maeno T, Ota F, Ueno M, Korekane H, Takamatsu S, Shirato K, Matsumoto A, Kobayashi S, Yoshida K, Kitazume S, Ohtsubo K, Betsuyaku T, Taniguchi N: J Biol Chem 287, 16699, 2012

2) Kamio K, Yoshida T, Gao C, Ishii T, Ota F, Motegi T, Kobayashi S, Fujinawa R, Ohtsubo K, Kitazume S, Angata T, Azuma A, Gemma A, Nishimura M, Betsuyaku T, Kida K, Taniguchi N: Biochem Biophys Res Commun 424, 112, 2012

3) Shirato K, Gao C, Ota F, Angata T, Shogomori H, Ohtsubo K, Yoshida K, Lepenies B, Taniguchi N: Biochem Biophys Res Commun. 435, 460, 2013

Session 5　Toward Drug Discovery from Glycoscience

Chair : Yasuhiro Kajihara (Osaka University)

Construction of drug discovery platforms using yeast

Yoko Yashiroda (RIKEN)

Identification of bioactive small molecules and their cellular targets is a primary task in their development for biological tools and therapeutics. We developed a high-throughput phenotypic screening strategy for drug discovery and a systematic method for target identification. We overexpressed the whole ORF library (ORFeome) of fission yeast and additionally 10,000 human cDNA clones in fission yeast and identified 5~10% of genes among the whole genome that caused growth defects, respectively. We have identified several new bioactive compounds capable of abating the growth defect phenotypes of gene-overexpression strains. Furthermore, based on functional chemical genomic profiling obtained by scoring budding yeast haploid deletion mutants for hypersensitivity to a set of chemical compounds, we have developed a barcode-sequenced method for identification of the molecular target and/or the target pathway. I will discuss the usefulness of these yeast systems for drug development.

References
1) Yashiroda et al.: BBRC 394, 569, 2010
2) Parsons et al.: Nat Biotechnol 22, 62, 2004.
3) Costanzo et al.: Science 327, 425, 2010

Synthesis of bisected N-glycan units and design of glycosyltransferase inhibitors

Shinya Hanashima (Osaka University)

The low-molecular-weight inhibitors for glycosyltransferases are a vital tool to investigate functions of glycans and glycosyltransferases. Furthermore, such inhibitors have a potential to be a new medicinal candidate due to the expanding correlations between glycan structures and certain diseases.

N-acetylglucosaminyltransferase(GnT)-V plays a vital role in tumor metastasis and malignant transformations. We designed and synthesized bisubstrate-type inhibitors having a minimum acceptor unit and UDP-GlcNAc together by connecting through alkyl linkers. Inhibition assay shows that the synthetic inhibitors have potent inhibitory activities to GnT-V in a linker length-dependent manner.

Recent findings suggest that GnT-III is involved in certain diseases including Alzheimer's disease. To determine the minimum acceptor structure, we systematically synthesized four units of the branching N-glycan moieties. The inhibition study suggests that GnT-III recognizes relatively large molecular surface on the acceptor

unit including core trimannose and GlcNAc at αl-3 branch.

Toward the use for *in vivo* inhibition studies, these synthetic probes might be required for further tuning in their higher molecular weight, hydrophilicity, and stability. In addition to these synthetic approaches, screening from compound library and determination of the protein-substrate complex structure would help finding for the efficient inhibitors of glycosyltransferases.

References
1) Hanashima S, Inamori K, Manabe S, Taniguchi N; Ito Y: Chem Eur J 12, 3449-62, 2006
2) Hanashima S, Korekane H, Taniguchi, N; Yamaguchi Y: Bioorg Med Chem Lett 24, 4533-7, 2014

Therapeutic method for malignant tumor using carbohydrate-mimetic peptide

Michiko Fukuda (AIST)

In the era of translational research, carbohydrate-based drug discovery has yet to be explored, due in part to our inability to undertake automated chemical synthesis of complex carbohydrates. This dilemma can be partially, if not totally, circumvented by using carbohydrate-mimetic peptides, which can be identified from peptide-displaying phage libraries. We previously identified a carbohydrate mimetic peptide designated IF7, that binds to Anxa1, an endothelial cell surface marker highly specific to malignant tumors. To determine whether IF7 could be used for drug delivery to malignant tumors, we injected fluorescence-labeled IF7 peptide into tumor-bearing mice and observed fluorescent signals in tumors within one minute of injection. When a conjugate of IF7 with an anti-cancer drug was injected intravenously into tumor-bearing mice, we observed tumors shrank at low drug dosage without side effects. Since IF7 enters endothelial cells via vesicle transport, we hypothesized that an IF7-conjugated drug could cross the blood brain barrier, the major obstacle to chemotherapy in patients with brain malignancies. Interestingly, when we performed intravenous injection of the IF7-conjugated drug into a mouse with two tumors, one subcutaneous and the other in brain, tumor growth was suppressed, regardless of location. These findings suggest the clinical relevance of carbohydrate mimetic peptide.

References
1) Fukuda MN: Peptide-displaying phage technology in glycobiology. Glycobiology 22, 318-25, 2012
2) Oh P, Li Y, Yu J, Durr E, Krasinska K M, Carver L A, Testa JE:, Schnitzer JE: Subtractive proteomic mapping of the endothelial surface in lung and solid tumour

for tissue-specific therapy. Nature 429, 629-35, 2004
3) Hatakeyama S, Sugihara K, Shibata TK, Nakayama J, Akama TO, Tamura N, Wong SM, Bobkov AA, Takano Y, Ohyama C, Fukuda M, Fukuda M N: Targeted drug delivery to tumor vasculature by a carbohydrate mimetic peptide. Proc Natl Acad Sc USA 108, 19587-92, 2100

Closing Remarks