The 11th Symposium
of Japan Consortium for Glycobiology and Glycotechnology

Lifestyle-Related Diseases and Glycoscience

October 25-26, 2013 at Tohoku Pharmaceutical University (Sendai)

Program October 25 (Friday), 2013

Opening Address
Jin-ichi Inokuchi (Organizing Chair, Tohoku Pharmaceutical University)

Greetings from Organizer
Naoyuki Taniguchi (JCGG President, RIKEN)

The Current State of the Master Plan Decision
Koichi Furukawa (Nagoya University)

Session 1　Metabolic Syndrome and Glycoscience

Chairs : Kazuo Nita (Tohoku Pharmaceutical University),
Hideyoshi Higashi (Tohoku Pharmaceutical University)

Deglycosylation in pancreatic beta cells in pathogenesis of type 2 diabetes
Kazuaki Otsubo (RIKEN)

In pancreatic beta cells, the process of glucose uptake from interstitial fluid by a passive and facilitative transport across the cellular plasma membrane is essential for insulin secretion. It has been elucidated that the cell surface expression of glucose transporter (GLUT) is attenuated in the early stage of diabetes that should be associated with failure of glucose-stimulated insulin secretion (GSIS) of beta cells, although the pathophysiological and molecular mechanism remained unclarified. We have revealed that GnT-IVa-dependent glycosylation of GLUT2 is essential for its cell surface residency by the formation of GLUT2 N-glycan-galectin lattice in pancreatic beta cell. Furthermore, the high-fat diet ingestion elevates blood free-fatty acids levels and induces substantial oxidative stress in pancreatic beta cells that causes nuclear exclusion of transcription factors regulating GnT-IVa expression, and consequently impairs GLUT2 glycosylation and GSIS. Moreover, we found that the GLUT2 N-glycan-galectin lattice anchors GLUT2 in non-lipid-raft microdomain. Dysglycosylation of GLUT2 abolishes the lattice formation and induces redistribution of GLUT2 into lipid-raft microdomains that causes an interaction with a lipid-raft protein, Stomatin, which attenuates the glucose transport activity. These results indicate that GLUT2 N-glycosylation dynamically regulates glucose sensor function

and its failure is a part of pathogenesis of type 2 diabetes.

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O-GlcNAc modification and diabetes

Yoshihiro Akimoto (Kyorin University)

Increased flux through the hexosamine biosynthesis pathway promotes the O-GlcNAcylation of proteins, which is a unique posttranslational modification of nuclear and cytoplasmic proteins and has been implicated in the development of insulin resistance and diabetes complications. In our previous immunohistochemical study, we demonstrated that the O-GlcNAcylation level increased in various tissues including kidney from diabetic GK rats, which is an animal model of type 2 diabetes. To identify marker proteins that change in their extent of O-GlcNAcylation in the diabetic kidney, we separated total kidney proteins by two-dimensional gel electrophoresis. O-GlcNAcylated proteins were identified by immunoblotting and peptide mass fingerprinting. O-GlcNAcylated proteins that changed significantly in the degree of O-GlcNAcylation were identified as cytoskeletal proteins (α-actin, α-tubulin, α-actinin 4, myosin) and mitochondrial proteins (ATP synthase, pyruvate carboxylase). Immunoprecipitation and immunoblot studies, as well as in situ PLA demonstrated that the extent of O-GlcNAcylation of the above proteins increased in the diabetic kidney.

In conclusion these results suggest that in the diabetic kidney the morphological changes in the glomerulus and tubules may be ascribed to the abnormal O-GlcNAcylation of cytoskeletal proteins including α-actinin 4.

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Glycosphingolipids in cell rafts: Their role in T cell development and immune diseases

Masakazu Nagafuku (Tohoku Pharmaceutical University)

T cell development depends on signals activated by TCR and many other receptors. These receptor-mediated signaling events begin at the cell surface, and therefore they must be influenced by lipid environment in the plasma membrane. The highly organized membrane microdomains or lipid rafts have been known to be enriched in cholesterol and sphingolipids (gangliosides and sphingomyelin) and are thought to act a platform for signaling. Importantly, rafts are heterogeneous in their specific sphingolipid content and different types of rafts co-exist with different physiological properties and functions at the plasma membrane. We have determined the expression profiles of gangliosides and sphingomyelin during the differentiation processes from immature to mature thymocytes in mice. The expression levels of both GM1 ganglioside and sphingomyelin were decreased with the progression of double negative cells and reached the bottom at double positive stage. Sphingomyelin expression levels were then markedly increased more than 10-folds in CD4 single positive (SP) cells but were comparable in CD8 SP cells. In contrast, GM1 ganglioside expression levels were increased in CD8 SP cells but were comparable in CD4 SP cells. The gene expression patterns involved in sphingolipid biosynthesis (Sphingomyelin, GM3 and GM2 synthases) coincided with the above-mentioned distinct expression profile of ganglioside and sphingomyelin during the differentiation processes. These results suggest unique rafts are formed in the plasma membrane during T cell development and these rafts provide the appropriate distinct intracellular signaling events for successful differentiation.

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Involvement of glycosphingolipid metabolism in maintenance and breakdown of homeodynamic state

Jin-ichi Inokuchi (Tohoku Pharmaceutical University)

A new concept "Life style-related diseases, such as type 2 diabetes, are a membrane microdomain disorder caused by aberrant expression of gangliosides" has arisen[1,2]. Adipogenesis of mesenteric preadipocytes is increased following depletion of the resident macrophages with concomitant decrease of glycosphingo-lipid (GSL) levels including GM3. Preadipocytes prepared from GM3 synthase KO mice differentiate much faster than normal cells. In the state of insulin resistance induced by inflammatory cytokines, GM3 synthase gene is elevated, and the

inhibition of GSL biosynthesis ameliorates the insulin resistance. Thus, GM3 is involved in the spatiotemporal regulation of adipogenesis, homeostasis of insulin signaling and the development of insulin resistance. To investigate systemic effects of gangliosides in metabolic syndrome, we developed the GM3S deficient mice with KK and KKAy backgrounds. Over-eating and excess BW gain of KKAy mice were completely suppressed in GM3S$^{-/-}$KKAy mice, while GM3S deficiency in KK mice didn't show any reduction of appetite and BW compared to the control KK mice. The selective suppression of KKAy specific diabetic phenotypes may be a result of normalization of hypothalamus energy regulatory systems. GM3S plays a key role in the development of peripheral insulin resistance and obesity-induced abnormal energy expenditure possibly in hypothalamus.

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Special Lecture I

Chair : Jin-ichi Inokuchi (Tohoku Pharmaceutical University)

Metabolic information highways for systemic regulation of glucose and energy metabolism

Hideki Katagiri (Tohoku University)

Metabolism is coordinated and regulated among different organs/tissues throughout the body. Communication among tissues is extremely important for maintaining systemic homeostasis. We have identified several neuronal networks which are involved in inter-organ metabolic communication.
1) Adipose expression of UCPI regulates appetite due to improving hypothalamic leptin sensitivity.
2) Hepatic overexpression of PPARγ, which induces lipid accumulation in the liver, reduces peripheral adiposity via increased basal metabolic rates. This inter-tissue network functions as an anti-obesity system. However, this homeostatic mechanism causes obesity related hypertension via sympathetic activation.
3) Hepatic ERK activation promotes pancreatic cell proliferation.
4) Hepatic overexpression of glucokinase, which enhances glucose metabolism in the liver, suppresses thermogenesis in brown adipose tissue. This energy-saving system at the whole-body level contributes to determining obesity predisposition

among murine strains.

Our findings indicate that the brain obtains a variety of metabolic information from peripheral tissues through neuronal information highways, resulting in cooperative metabolic regulation among peripheral tissues. On the other hand, these network systems ironically cause features of the metabolic syndrome, such as hypertension, hyperinsulinemia and obesity itself, under continuous excess energy intake. Thus, our discoveries may highlight the importance of the brain function in maintaining metabolic homeostasis and developing metabolic diseases.

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Special Lecture II

Chair : Tamao Endo (TMIG)

Physiological roles of fatty acid binding proteins (Fabps)

Noriko Osumi (Tohoku University)

Both genetical and environmental factors independently and synergistically play important roles in development, maintenance, and aging of the central nervous system. We have been studying the function of Pax6 transcription factor that is a key regulator in the developing brain and in the visual and olfactory systems. During the course of elucidating downstream functional molecules of Pax6, we have identified a fatty acid binding protein Fabp7 working in neural stem/progenitor cells and in astrocytes. We reveal that Fabp7 is critical in maintaining neural stem/progenitor cells to proliferate in the embryonic and postnatal hippocampal neurogenesis. From genetical analyses of Japanese patients, a unique SNP of FABP7 gene is associated with onset of schizophrenia, and Fabp7 knockout (KO) mice actually exhibit impairment in the sensorimotor gating system, a typical endo-phenotype of schizophrenia. In the inner ear, glial cells and supporting cells express Fabp7. Curiously enough, we have recently demonstrated that Fabp7 KO mice show gene-dosage dependent anti-aging phenotypes in age-related hearing loss at the functional and morphological levels. We are wondering whether the superficial discrepancy of schizophrenic phenotypes and of anti-aging phenotypes due to loss of Fabp7 functions has significance in the evolutional point of view.

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Program October 26 (Saturday), 2013

Session 2 Cancer and Glycoscience

Chairs : Taeko Miyagi (Tohoku Pharmaceutical University)
Jiango Gu (Tohoku Pharmaceutical University)

Clinical application of new assay system detecting cancer-associated aberrant glycosylation on prostate specific antigen (PSA)

Chikara Ohyama (Hirosaki University)

PURPOSE: To develop an assay system to measure prostate specific antigen (PSA) with cancer-associated aberrant glycosylation and to compare the diagnostic accuracy with conventional PSA test.

MATERALS AND METHODS: We previously identified prostate cancer (PCa)-associated aberrant glycosylation on PSA, that is, α2,3-linked sialylation as an additional terminal N-glycan on free PSA (S2,3PSA). In the present study, we developed a new assay system for measurement of S2,3PSA with magnetic microbead-based immunoassay. We used the magplex beads to measure serum S2,3PSA, employing anti-human free PSA monoclonal antibody (8A6) for coating beads and anti-α2,3-linked sialic acid monoclonal antibody (HYB4) for detection. The amount of serum S2,3PSA was represented as a mean fluorescence intensity (MFI) value. We first determined cut-off value through a pilot study, and then measured serum S2-3PSA from biopsy-proven 314 individuals including 138 patients with PCa and 176 patients with non-PCa whose PSA was lower than 10.0 ng/mL.

RESULTS: We determined MFI cut-off value of I130 with a specificity of 72.0% and sensitivity of 95% for diagnosis of PCa. In the validation study, area under the curve (AUC) for detection of PCa with S2,3 PSA was 0.84 which was significantly higher than those with PSA (0.57). ($P<0.001$)

CONCLUSION

Although the present study is small and preliminary, these results suggest that detection of serum S2,3PSA with magnetic microbead-based immunoassay may improve accuracy of early detection of prostate cancer. Further validation study is warranted.

GOLPH3 regulates N-glycosylation of integrin and cell adhesion

Jiango Gu (Tohoku Pharmaceutical University)

Recently, a Golgi protein, Golgi phosphoprotein 3 (GOLPH3) was identified as a new oncogene that is commonly amplified in human cancers. To investigate whether the expression of GOLPH3 was involved in the N-glycosylation processes in mammalian cells, and affected cell behaviors, we performed a loss-of-functional

study. Cell migration on fibronectin or laminin was suppressed in GOLPH3 knockdown (KD) cells, and the suppression was restored by re-introduction of GOLPH3 gene. Interestingly, N-glycosylation status of β1 integrin obtained from KD cells was apparently different from those in control cells, while it was normalized in the restored cells. HPLC and LC/MS analysis showed that the sialylation levels of N-glycans were specifically decreased in KD cells. To explore the molecular mechanism for the specific effect on sialylation by GOLPH3 expression, we examined the interactions between glycosyltransferases and GOLPH3, and found GOPLH3 specifically associated with sialyltransferases. Furthermore, the cytoplasmic tails of sialyltransferases were shown to be important for the association. Taken together, these results suggest that GOLPH3 regulates glycosylation status of integrin and its biological functions, which may give a new insight for the functions of GOLPH3 in cancer.

A challenge of glyco-science to pancreatic cancer

Eiji Miyoshi (Osaka University)

Pancreatic ductal carcinoma (PDAC) is one of the most miserable diseases, which show extremely poor prognosis. The reason why PDAC prognosis is poor depends on the difficulty of an early diagnosis and early recurrence after the operation. To overcome a variety of clinical problems in PDAC, an innovative strategy to develop a novel system of diagnosis and therapy is required. We have established a lectin-ELISA kit for fucosylated haptoglobin, which might be involved in micro/macro liver metastasis of PDAC, and identified small lipid complexes as a novel type of CA19-9 carrier molecules. Furthermore, we have developed porcine-type oligosaccharide vaccine to treat PDAC in animal models. In this symposium, we would like to introduce our recent and future studies of glycol-medicine for PDAC.

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epitopes: A novel approach to immunotherapy in pancreatic cancer. Cancer Res. 70(13), 5259-69, 2010

Roles of sialidase in carcinogenesis

Taeko Miyagi (Tohoku Pharmaceutical University)

Altered sialylation is closely related to malignant properties, including invasiveness and metastatic potential. To elucidate the underlying molecular mechanisms, our studies have focused on mammalian sialidase, which catalyzes the removal of sialic acid residues from glycoproteins and glycolipids. Among the four types of mammalian sialidase (NEU1-4) identified to date, we previously demonstrated that the plasma membrane-associated sialidase NEU3, a key enzyme for ganglioside degradation, is markedly up-regulated in various human cancers. This aberrant up-regulation was found to cause acceleration of malignant properties, including promotion of cell invasion and motility and cell survival of cancer cells probably through activation of EGFR signaling. Here, we investigate whether and how NEU3 contributes to cancer initiation as well as progression by analyzing cancer stem-like characteristics and tumor initiating capability in colon cancer cells, and uncover a close link between NEU3 and Wnt/β-catenin signaling. These results suggest that NEU3 plays a crucial role in tumor initiation and progression through modulation of gangliosides by regulating the signaling pathways essentially involved in carcinogenesis. Thus, NEU3 may be a potential new target for cancer therapy.

Luncheon Seminar (sponsored by Shimadzu Co.)

New aspect revealed by targeted glycolipidomics

Akemi Suzuki (Tokai University)

Ceramide structures of glycolipids play critical roles in microdomain formation and functions, but characterization of their structures has not been easily accomplished because of the lack of sufficient analytical methods. LC-IT-MS gives an excellent clue for this difficulty. The method is applicable even for crude mixtures of glycolipids, providing information on sphingosine and fatty acid structures of ceramides as well as sequences of carbohydrate chains. Now, to accelerate functional studies of glycolipids, we need a data base compiling MS^2 and MS^3 spectra and in addition an efficient search engine for predicting their structures. This talk introduces present status of our activities and the results of actual analysis of glycolipids using crude fractions prepared from biological materials.

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Session 3　Neural Functions and Glycoscience

Chairs: Yasuhiro Hashimoto (Fukushima Medical University)
Hiroshi Kitagawa (Kobe Pharmaceutical University)

Roles of sulfated glycans in axonal regeneration

Kenji Kadomatsu (Nagoya University)

Biopolymers in the human body belong to three major classes: polynucleotides (DNA, RNA), polypeptides (proteins) and polysaccharides (glycans). Thus, important biological functions are expected to be attributable to glycans, but the significance of glycans remains largely unexplored in the nervous system (Fig). Axonal regeneration/sprouting after injuries does not easily occur in the adult mammalian central nervous system. This is due to the low intrinsic potential of regeneration and the emerging inhibitory molecules. The latter include the sulfated long glycans, chondroitin sulfate (CS) and keratan sulfate (KS). We found that enzymatic ablation of CS or KS, and genetic ablation of KS promote functional recovery after spinal cord injury. Interestingly, the combination of CS and KS ablations exhibits neither additive nor synergistic effects. Our findings indicate that KS and CS work in the same pathway in inhibition of axonal regeneration/sprouting. Elucidation of the mechanisms of action for KS and CS will pave the way to treatments to promote network rewiring and plasticity after neuronal injuries.

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Roles of chondroitin sulfate in the nervous system
Hiroshi Kitagawa (Kobe Pharmaceutical University)

Chondroitin sulfate (CS), a major component of the brain extracellular matrix, is reported to play a role in controlling critical period plasticity. Digestion of CS chains has shown to restore visual cortical plasticity in adult animals, but the underlying mechanisms remain unknown. CS chain is a linear polysaccharide consisting of repeating disaccharide units that can be substituted with sulfate groups at various positions, thereby producing characteristic sulfation patterns. Thus we hypothesized that sulfation patterns of CS chains regulate the critical period plasticity. Using transgenic mouse model overexpressing chondroitin 6-sulfotransferase-1, we found that a developmental increase in the 4-sulfation/6-sulfation (4S/6S) ratio of CS chains leads to the termination of the critical period in the mouse visual cortex. Condensation of CS-proteoglycans into perineuronal nets that enwrapped parvalbumin-expressing interneurons (PV-cells) was prevented by cell-autonomous overexpression of chondroitin 6-sulfation. Mechanistically, the increase in the 4S/6S ratio was required for accumulation of Otx2, a homeoprotein that activates development of PV-cells, and for functional maturation of electrophysiological properties of these cells. Our study reveals a novel mechanism for the critical period plasticity, in which specific sulfation patterns of CS chains regulate the maturation of PV-cells through the incorporation of Otx2.

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The Functional role of N-glycans expressed on glutamate receptors
Shogo Oka (Kyoto University)

AMPA-type glutamate receptors mediate most of the fast excitatory synaptic transmission in the mammalian brain and control synaptic strength. The regulated trafficking of AMPA receptors to the postsynaptic membrane is thought to be a major mechanism contributing to long-lasting changes in synaptic strength, including long-term potentiation (LTP) and long-term depression (LTD). AMPA receptors are tetrameric channels assembled from the subunits GluA1-GluA4, and all subunits have 4-6 potential N-glycosylation sites in their extracellular domains. In mature pyramidal neurons, GluA1 and GluA2 are predominantly expressed, and the great majority of AMPA receptors contain GluA2 subunits. We have demonstrated that the HNK-1 carbohydrate, one of the most characteristic glycol-epitopes in the nervous system, is specifically expressed on GluA2 and plays crucial roles in synaptic

plasticity. However, few studies have focused on the function of N-glycan itself in AMPA receptors. In this study, we obtained the data that N-glycans expressed on AMPA receptors have potential roles in synaptic plasticity

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"Brain type" N-glycan and CNS diseases
Yasuhiro Hashimoto (Fukushima Medical University)

Two transferrin (Tf) isoforms were found in human cerebrospinal fluid (CSF); one had α2,6 sialic acid-terminated N-glycans like serum Tf, whereas the other had biantennary asialo- and agalacto-complex type N-glycans (GlcNAc-terminated glycans) with bisecting β1,4GlcNAc and core α1,6fucose. The latter glycan appeared to be unique to central nervous system (CNS). We enriched GlcNAc-terminated glycoproteins from CSF by lectin affinity column chromatography. Their core proteins were identified by peptide mass fingerprinting. The proteins identified were screened for marker of CNS diseases such as neurodegenerative disorders and autoimmune diseases. Screening results will be discussed in our presentation.

Session 4　Applied Glycoscience
Chairs : Tadao Saito (Tohoku University)
Shin-ichiro Shoda (Tohoku University)

A new method for synthesis of oligosaccharide lipid derivatives
Shin-ichiro Shoda (Tohoku University)

Supplying alkyl glycosides is one of the most important roles of glyco-chemists because these compounds can create their own demands as useful building blocks for construction of complex glyco-materials. The most simple route for synthesis of unprotected alkyl glycosides was first reported by Emil Fischer.

Despite the great utility for carbohydrate synthesis, the use of a strong acid has hampered the applicability of Fischer protocol. In case of employing oligo-saccharides as starting sugars, acetal exchanges of the inner glycosidic bonds would occur as a result of intermolecular protonations by the acid catalyst to the glycosidic oxygens. In order to avoid these side interactions, a site-specific intramolecular activation at the reducing end is indispensable.

A convenient protection-free synthetic route toward alkyl glycosides has been established. The alcoholysis of one-step preparable glycosyl donors, 4,6-dibenzyl-oxy-1,3,5-triazn-2-yl (DBT) glycosides, under hydrogenolytic conditions gave the

corresponding glycosides in good yields without adding any acid promoters. The method could be successfully applied to glycosylation of oligosaccharides and long alkyl chain alcohols, giving rise to the corresponding oligosaccharide glycolipid derivatives. The present method would pave a way toward the glycosylation of acid labile oligosaccharides as well as acid labile aglycon alcohols in the synthetic field of carbohydrate chemistry.

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Trends and prospects for chemoenzymatic homogenous glycoprotein synthesis

Takashi Shirai (Noguchi Institute)

Some homogeneous glycoproteins are chemoenzymatically synthesized. One powerful approach is enzymatic remodeling. Initially heterogeneous glycoform mixtures are treated with an endoglycosidase (ex. Endo-M, Endo-S) to obtain GlcNAc-containing protein as an acceptor. Secondly homogeneous sugar oxazoline is prepared as a donor. Subsequent enzyme (ex. Endo-M(N175Q), Endo-S(D233Q))-mediated glycosylation to the exposed GlcNAc residue containing protein can then produce a homogeneous glycoprotein. These three key technologies (endo-glycosidase, sugar oxazoline, donor sugar) are established by Japanese researchers except Endo-S. So far mammalian cell culture systems are predominantly used for production of therapeutic glycoproteins. Here we propose "chemoenzymatic homogeneous glycoprotein synthesis" as an alternative production system for biopharmaceuticals which are completely free of animal cell culture system. In addition to three key technologies. We employed transgenic silkworm cocoons for production of targeted glycoprotein which would be trimmed to as an acceptor GlcNAc-containing protein. Our recent progress will be reported and future perspective will be discussed.

Reference
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Screening technology of probiotics with recognition and binding ability to the sugar chains of human colonic mucins and its application to the

prevention of colon cancer and/or ulcerative colitis

Tadao Saito (Tohoku University)

A new evaluation system to select probiotic lactic acid bacteria (LAB) with adherence to human colonic mucin (HCM) by recognizing sialic acid and/or sulfate on ABO-blood type antigens. LAB strains showing strong adhesion to human blood type-A antigen expressed on the intestinal mucosa were selected from 237 probiotic strains using the biosensor BIACORE 1000. A similar method was used to select harmful bacteria that also recognizing the same antigen in the human intestine. *Fusobacterium varium* was detected as one of harmful bacteria from the patient of ulcerative colitis (UC). Using a competitive adhesion experiment with a micro plate coated with the type-A antigen and the harmful bacteria, the bacteria was significantly decreased when they were added in combination with the type-A LAB. Our data suggested the blood type LAB strains may competitively inhibit pathogenic infections where this strategy may be useful in the development of improved future probiotic foods to protect human health.

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Synthesis and biological activities of non-hydrolyzable ganglioside analogs

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Gangliosides are glycosphingolipids that are ubiquitous components of mammalian cell membranes. GM3 was known to inhibit cell proliferation and auto-phosphorylation of EGFR stimulated by EGF. Plasma membrane-associated human sialidase NEU3 is known to hydrolyze the glycosidic linkage of sialic acid in GM3 to produce lactosylceramide, and is up-regulated in various cancer cells and tissues. Recent reports suggested that enzymatic metabolism of GM3 by NEU3 may be involved in cancer malignancy. To clarify the relationship between GM3 metabolism

and cancer malignancy by NEU3, we designed sialidase-resistant GM3 analogues with CF, CHF, and CH_2-sialoside linkage, in which the oxygen atom of O-sialoside linkage is replaced by the CF_2, CHF, or CH_2 group. We succeeded in the stereo-selective synthesis of these non-hydrolyzable GM3 analogues. For the construction of the key C-sialoside linkages, stereoselective Ireland-Claisen rearrangement reaction was used as a key step. Detail of the synthesis of these GM3 mimic molecules and their biological activity will be presented.

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Closing Remarks