Panel Discussion

Research trends on glycoproteins in the last five years

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Professor Ken Kitajima received his BSc and MSc degrees in biophysics and biochemistry at the University of Tokyo, Japan in 1982 and 1984, respectively, and obtained his Ph.D. from the University of Tokyo in 1987, where he determined overall structures of tandem-repeated peptides with highly polysialylated glycans for rainbow trout polysialoglycoprotein and discovered its processing enzyme that are activated concomitantly with cortical vesicular exocytosis at fertilization. From 1988-1989, he was a postdoctoral fellow at the University of Tokyo with Professor Yasuo Inoue, and in 1989, became the Assistant Professor at the University of Tokyo. In 1996, he moved to Nagoya University as Associate Professor of Graduate School of Bioagricultural Sciences. In 2000 he became the Associate Professor of the Bioscience and Biotechnology Center, Nagoya University, and since 2004, has been the Professor of the same center. He is currently a member of the Editorial Boards of Glycobiology, Glycoconjugate Journal, and the Journal of Biological Chemistry. He was also a National Representative of the International Glycoconjugate Organization until 2017. He is a Board of Trustees of the Japanese Society of Carbohydrate Research since 2007. In his laboratory, three lines of research work have been going on: (I) Sialic acid metabolisms in health and disease, currently focusing on biosynthetic mechanisms of deaminoneuraminic acid (Kdn) and sulfated sialic acid and on significance of sialic acid metabolism-related enzymes at animal level using medaka fish; (II) Structural diversity and novel functions of polysialic acids in vertebrate brains and sea urchin gametes; (III) Biological significance of membrane microdomains as a site of the glycan-mediated interactions.

Omics in glycoscience

Biochemical and molecular biological approaches have been successful in describing mechanisms for various biological phenomena with a focus on specific molecules. On the other hand, for the last decade, methodology and concept of life science have a great deal changed. As an extension of conventional approaches, a different discipline of research has been lately focused on, i.e., systems biology, which aims at understanding a whole animal on the basis of integrated information from various omics, including transcriptomics, proteomics, and metabolomics. Irrespective of the current state of successful integration of various omics in systems biology, each omics is practically used for identification of important molecules in specific stages or organisms. In research fields of glycobiology, glycomics is now acknowledged as an ordinary methodology, as is the case with proteomics in life science [1]. However, for the last few years, glycoproteomic analysis, which clarifies the glycoform of every glycosylation site on specific proteins, has been developed, and is increasingly used now, because it gives much more useful information than simple glycomics [2]. Metabolomics has been established for describing whole metabolites in a specific state of cell or an individual organism. One of important metabolites in glycobiology is a group of nucleotide sugars that are used as donor substrates for various glycosyltranferases [3]. The hexosamine biosynthetic pathway, through which UDP-GlcNAc is synthesized from Fructose 6-phosphate, a metabolite of the glycolysis, and glutamine, a protein metabolite, has been successfully studied

for biosynthetic regulation of *N*- and *O*-glycans of proteins and *O*-GlcNAcylation of regulartory proteins in cytosol and nucleus [4].

Genome wide association study in glycomedicine

In addition, recent technical innovation of nucleotide sequencing has provided us with the whole genome sequence of any organism in several hours, and genome sequences from an extremely large number of individual samples are now available. The genome wide association study (GWAS) for the relationship between the frequency of the single nucleotide polymorphisms (SNPs) and certain diseases using those data has been performed lately and is still expanding now. GWAS data can be used in various ways, and is especially powerful in quantitative trait loci (QTL) analysis. A significant relationship between schizophrenia and the polysialyltransferase ST8SIA2/STX gene was reported [5], and it has been shown that impaired properties of resultant polysialic acid structures on the neural cell adhesion molecule (NCAM) raise a risk for developing schizophrenia [6]. So called "big data" arisen from omics and GWAS data need to be efficiently used. Therefore, it is a natural consequence that significance of bioinformatics is more and more recognized. Many efforts are now being made by many researchers of informatics, including bioinformatics of glycobiology [7].

Genome editing technologies and glycoscience

Advances in genetic modification technologies have brought about drastic changes of research style in life science. Through the technologies, we can pursue a role of a specific gene in a specific time and space. RNA interference and morpholino antisence oligonucleotide technologies are currently used as a standard method. More recently, genome editing technologies such as the transcription activator-like effector nuclease (TALEN) and the clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated protein 9 (CRISPR-Cas9) has emerged and has been more frequently used for a few years. Using CRISPR-Cas9 technology, we can obtain cells or animals with the disrupted target gene. It is prominent that these technologies enable us to make genetic modification in basically any organisms. Using not only mouse but also small fish, significance of unusual glycosylation of dystroglycan has been confirmed.

Discoveries of new glycan structures

Finally, most important features of glycobiology, in my opinion, are those discoveries of new glycan structures, characterization of highly ordered glyco-complex with proteins and lipids, such as lipid rafts and exosomes, and understanding of complex biosystems such as microbial flora, which are not directly related to the up-to-date style of life science described above. With regard to protein glycosylation, extremely novel glycan structures of α-dystroglycan have been demonstrated, in which the Xyl/GlcA repeat, (-3Xylα1-3GlcAβ1-)n, and CoreM3 structure, GalNAcβ1-3GlcNAcβ1-4 (phosphate-6) Manα1-Ser/Thr, are linked by a novel tandem Rbo5P (ribitol 5-phosphate) structure [8]. This type of *O*-mannosyl glycan on α-dystroglycan is required for its binding to extracellular matrix components. Recently, a novel *O*-Man modification on E-cadherin was reported in mammals, and has been shown to be independent of known protein *O*-mannosyltransferases 1 and 2 that modify α-dystroglycan [9]. A novel *O*-mannosylation process on the large cadherin superfamily is predicted in mammalian cells. *O*-GlcNAcylation of cytosolic and nuclear proteins is recognized as the common protein glycosylation that occurs inside the cell. In contrast, *O*-GlcNAc modification on extracellular protein domains has recently been found as a novel example for protein glycosylation [10], which regulates Notch signaling and vascular development.

Future perspectives

Current tendency to use glycoproteomics rather than glycomics will be continued, and GWAS for the relationship between SNPs and disease glycoproteomics will be frequently carried out. Use of CRISPR-Cas9 technology to examine function of a specific gene at cellular and animal levels will be popular in the glycobiology field, although, at the same time, we have to carefully think about the situation that the technology of gene manipulation is expanding, irrespective of the ethical problems. Those up-to-date technologies will enable us to understand in detail the relationship between a specific gene and phenotypes related glycan chains, and will be able to identify unknown glycosyltransferases and their regulatory proteins not only in model animals, but also in various animals other than experimental animals. We should continuously make efforts to find novel forms of glycosylation, to understand mechanisms underlying biological phenomena by characterizing functional complex of glycans, proteins, and lipids, and to understand roles of various microbial flora in terms of human health and disease. There are still more subjects than we can solve, as has been the case so far. Diversity must be more and more important in research subject of glycobiology.

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