Panel Discussion

Trends of Glycolipid Research that Emerged from Analysis of Grant Applications for Mizutani Foundation for Glycoscience

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Makoto Ito received his Ph.D. in 1981 from Kyushu University, Fukuoka, Japan, under the supervision of Prof. Manabu Kitamikado. He studied an endo-β-galactosidase capable of cleaving the internal β-galactosidic linkage in keratan sulfate as well as lacto/neolacto-series glycosphingolipids in his thesis study. Between 1982 and 1993, he worked at the laboratory of Glycoconjugate Research (Dr. Tatsuya Yamagata) at the Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan, first as a postdoctoral fellow, then as a senior researcher. Dr. Ito discovered the endoglycoceramidase that cleaves the β-glycosidic linkage between oligosaccharide and ceramide of various glycosphingolipids at this institute. He received the Young Scientist Award for Biochemists from the Japanese Biochemical Society in 1991. He then returned to Kyushu University in 1993 as an associate professor. He cloned and characterized many novel enzymes involved in metabolism of glycolipids and sphingolipids, including sphingolipid ceramide *N*-deacylase (SCDase), neutral ceramidase (from bacteria to humans), human cytosolic glucocerebraosidase (Klotho-related protein, GBA3), endogalactosylceramidase (EGALC), fungus glucocerebrosidase and sterylglucosidase (EGCrP1 and EGCrP2, respectively), glycolipid-specific β-*N*-acetylgalactosaminidase (NgaP), and zebrafish GM4 synthase. He was appointed a full professor in 2002 and is currently a professor of Molecular Bioscience, Department of Bioscience and Biotechnology, Faculty of Agriculture, and a professor of Bio-Architecture Center (BAC), Kyushu University. He served as a president of the Japanese Society of Carbohydrate Research from 2015 to 2017, and as a director of the Japanese Biochemical Society from 2017 to 2019. He is interested in the metabolism and functions of glycolipids from bacteria to humans.

Glycolipids are complex carbohydrates in which monosaccharides or oligosaccharides are linked to lipids. Depending on the type of lipid, they are mainly classified as sphingoid-based glycosphingolipids, glycerol-based glycoglycerolipids, and sterol-based sterylglycosides. In addition to these glycolipids, GPI-anchored proteins are sometimes regarded as glycolipids. Glycosphingolipids are ubiquitous components of plasma membranes of vertebrates, invertebrates, plants, yeasts, and some bacteria while glycoglycerolipids are mainly present in plants and bacteria especially abundant in Archaea. The latter are rare in mammals but a sulfated glycoglycerolipid named seminolipid is the most abundant glycolipid in the testis and sperm. Sterylglycosides are major glycolipids in plants and fungi; however, they are also present in mammals.

Every year there are over 150 applications to the Mizutani Foundation from all over the world. In this symposium, the author will report and discuss the trends of glycolipid research that have been analyzed from Grant applications over the past three years. Applications targeting glycolipids are approximately 15 to 20% of all applications. This trend has not changed significantly in the past three years. The most common theme is immunity/infectious diseases, followed by themes such as metabolism, neuron/brain functions, lysosomal storage diseases, and chemical synthesis (**Figure 1**). Given the classification of glycolipids, the most common theme is related to glycosphingolipids, but those targeting glycoglycerolipids and sterylglycosides are also found.

Until recently, almost all enzymes involved in the synthesis and degradation of glycosphingolipids have been identified in mammals, including humans. Knockout mice of individual genes have been generated and analyzed. Deficiency of synthases that form the core structure of glycolipids, such as glucosylceramide and lactosylceramide, has decisive effects on early mouse development and leads to death in the fetal stage. However, the molecular mechanism of why they died because of the lack of these synthases is not clear. Zebrafish and medaka can be used to visualize the early development process, and may be useful in clarifying how these glycolipids play a role in early developments. Analyses of the GM3 synthase knockout mice revealed that this simple ganglioside is important for maintaining the normal function of the lipid microdomain and various membrane receptor functions. Failure of GM3-mediated cellular functions may exhibit severe diseases such as diabetes and hearing loss [1]. On the other hand, GM3 only mice have been found to cause neurodegeneration due to complement activation and inflammation, suggesting that the normal ganglioside composition is critical for neural tissue integrity and repair [2].

With respect to immunity/infectious diseases, research on how the glycolipids of pathogens activate the host's innate immune system is an important and interesting topic. It is reported that Mincle (macrophage-inducible C-type lectin) uses glycolipids as ligands, and activates the host innate immune system, e.g., Mincle recognizes a glycolipid called trehalose dimycolate of *Mycobacterium tuberculosis*, which is the causative bacterium of tuberculosis. Very recently, glucosyl diacylglycerol of *Streptococcus pneumoniae* has also been shown to be a potent ligand of Mincle [3].

In recent years, lipid rafts consisting of sphingolipid and cholesterol has been actively studied. Lactosylceramide is enriched in the specific microdomain of mouse neutrophils and mediates the cell migration and phagocytosis [4]. It was shown that there is a new type of microdomain that constitutes phosphatidyl glucoside in brain cells. Since this unique glycolipid is composed only of saturated fatty acids, it phase-separates from the phospholipid bilayer consisting of unsaturated fatty acids and forms a specific lipid microdomain. Lyso-form of phosphatidyl glucoside, a metabolite of phosphatidyl glucoside, regulates modality-specific sensory axonal induction of the spinal cord [5]. With regard to chemical synthesis, progress has been made in the synthesis of chemical probes that enable visualization of glycolipid-rich microdomains.

The lysosomal degradation of glycosphingolipids is performed by a combination of lysosomal glycosidases and noncatalytic activator proteins and, in some cases, specific anion phospholipids such as bis(monoacylglycero)phosphate. Lysosomal storage diseases are caused by a deficiency of one of the glycosidases or activator proteins. GBA1 is an acid β-glucocerebrosidase in lysosomes. The deficiency of GBA1, a causative gene of Gautier's disease, results in the accumulation of SNCN/α-synuclein as well as GlcCer, and increases the risk of Parkinson's disease [6]. Inhibition of the autophagy pathway by inactivation of protein phosphatase 2A may be one important scenario; however, its molecular mechanism is not well understood. Interestingly, GBA1 and GBA2 synthesize a cholesteryl glucoside using glucosylceramide and cholesterol as donor and acceptor substrates, respectively [7]. However, the functional relationship between cholesteryl glucoside and these diseases is not clear at present.

Lyso-forms of glycosphingolipids are detected in normal tissues and cells at very low levels; however, they accumulate in tissues of individuals with inherited sphigolipid storage diseases and may contribute to the dysfunction of various cellular events including cytokinesis [8]. The enzyme (SCDase) that produces lyso-foms of glycosphingolipids has been found in bacteria but not in mammals. Very recently, acid ceramidase was found to be involved in the formation of lyso-forms of glycosphinoglipids in lysosomes [9].

Regarding the structural analysis of glycolipids, the recent progress of mass spectrometry is remarkable. Although more than 200 glycosphingolipids with structurally distinct glycans are known, ceramide moieties are also quite heterogeneous in structures. The comprehensive profiling glycosphingolipid glycans using mass spectrometry after cleaving ceramide moiety by endoglycoceramidase is shown to be useful for quantitative cellular glycomics [10].

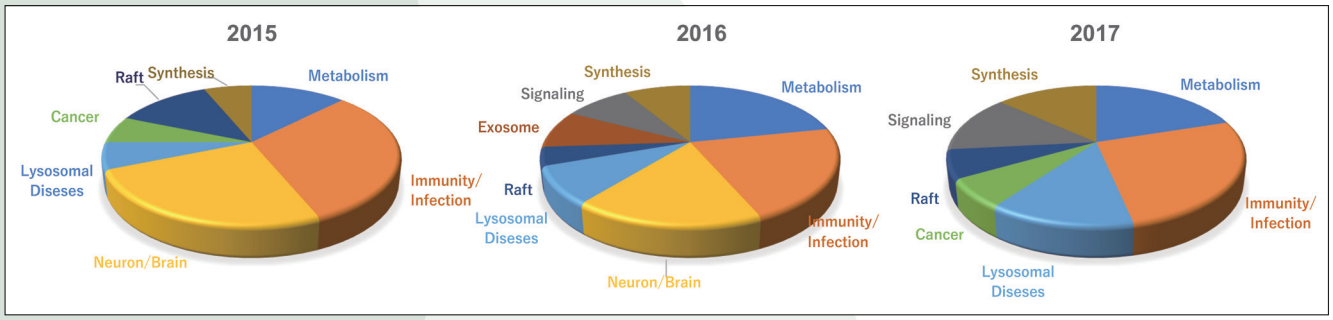

Figure 1
Classification of subjects of glycolipid research in grant applications for Mizutani Foundation for glycoscience from 2015 to 2017.

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