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Integrative Roles of Gangliosides in the Nervous System: Novel Molecular Mechanisms Elucidated by Genetic Engineering

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Abstract: Acidic glycosphingolipids, gangliosides are highly and consistently expressed in nervous tissues of vertebrates at high levels. Therefore, they have been believed to be largely involved in the development and function of nervous systems. Recent studies with genetic engineering of glycosyltransferase genes have revealed novel aspects of roles of gangliosides in the regulation of nervous tissues with substantial basis. In this review, novel findings on the ganglioside functions and their modes of action elucidated mainly by studies of gene knockout mice have been summarized. In particular, roles of gangliosides in the regulation of lipid rafts to maintain the integrity of nervous systems have been reported with a focus on the roles in the regulation of neuro-inflammation and neurodegeneration via complement

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systems. In addition, recent advances in the studies of congenital neurodisorders due to the genetic mutaions of ganglioside synthase genes, and also in the techniques for the analysis of ganglioside functions have been introduced.

Keywords: ganglioside; knockout; neurodegeneration; glycosphingolipid; inflammation; microdomain

1. Introduction

Nervous tissues are differentiated from ectoderm, and outline of their morphology is determined until birth. During the growth stage after birth, further functional differentiation proceeds, and fundamental shapes and functions are established within several years in the case of humankind. However, formation of functional networks based on various experiences and learning activities keep proceeding continuously, leading to the maintenance of high-grade nerve functions due to the plasticity of nervous systems [1]. After reaching middle age, network function gradually decline along with aging, and physiological and pathological degeneration gradually proceed in nervous tissues [2]. Under definite pathological conditions, marked tissue degeneration is induced, leading to functionally irreversible states such as occurrence of dementia. During individual processes of natural history of nervous systems, structures of carbohydrates on proteins and lipids seem to dramatically alter [3,4] and be involved in the formation of appropriate environments for the high-grade structures and functions based on the molecular modification required for each step.

Since acidic glycosphingolipids, gangliosides are highly and consistently expressed in nervous tissues of vertebrates at high levels [3], huge contribution of them in the neurological function have been long expected [5,6]. Actually, the fact that ganglioside expression patterns dramatically change during development has suggested that they play critical roles in the evolution and differentiation of nervous systems [7,8]. On the other hand, for carbohydrates on proteins, it appears that sufficient understanding has

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not been obtained in terms of integrative analysis of nervous system-specific carbohydrate functions, although there have been a number of reports on the carbohydrate functions on individual proteins.

In this review, outline of functions of gangliosides recently obtained has been summarized with focus on the findings from studies of knockout mouse of various glycosyltransferase genes.

2. Roles of gangliosides

Gangliosides are sialic acid-containing glycosphingolipids, and widely expressed in almost all tissues and cells of vertebrates. They are enriched in brain tissues, suggesting that they are involved in the evolution and regulation of nervous systems [9]. The most intriguing feature of gangliosides is that they consist of hydrophilic carbohydrates and hydrophobic lipid portions [10], being expressed on the cell membrane and present in the out layer of lipid two layers as shown in Figure 1.

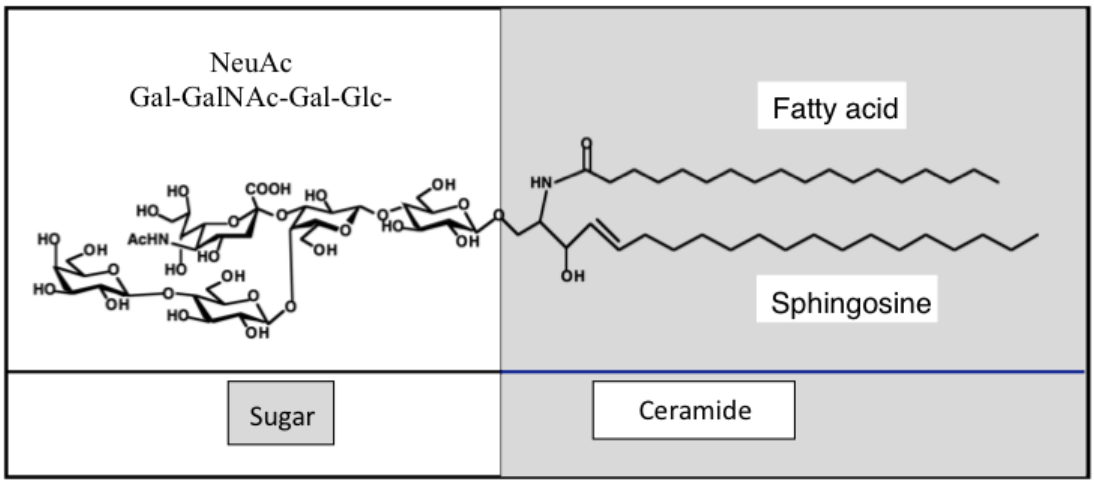

Fig. 1. Glycosphingolipids are amphipathic molecules, and expressed in the out layer of lipid two layers of cell membrane. Hydrophobic portion (ceramide) is embedded in the outer layer of the membrane, and sugar portion is protruding outside of the membrane.

Therefore, it has been a mystery how gangliosides are involved in the regulation of signals for cell differentiation, activation and malignant transformation [11]. In this review, ganglioside expression in the inflammation and neurodegeneration was summarized, and their roles in the maintenance of integrity and generation of

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phenotypes of cells with focus on the inflammation and degeneration due to the altered gangliosides were summarized.

2-1. Gangliosides in development and growth

The fact that ganglioside composition in brain tissues varies along with development and growth of organism has been well known in many reports [9,12]. As well understood, simple structure gangliosides such as GM3 and GD3 mainly exist at the initial stage of developments, i.e. embryonal day 12-14 (E12-14) of mice. At the differentiation stage after E16, when extention of neuritis and synapse formation occur, mature type gangliosides such as GM1, GD1a, GD1b and GT1b increase and become main components of brain tissues [13]. Main pathway of ganglioside synthesis is shown in Figure 2.

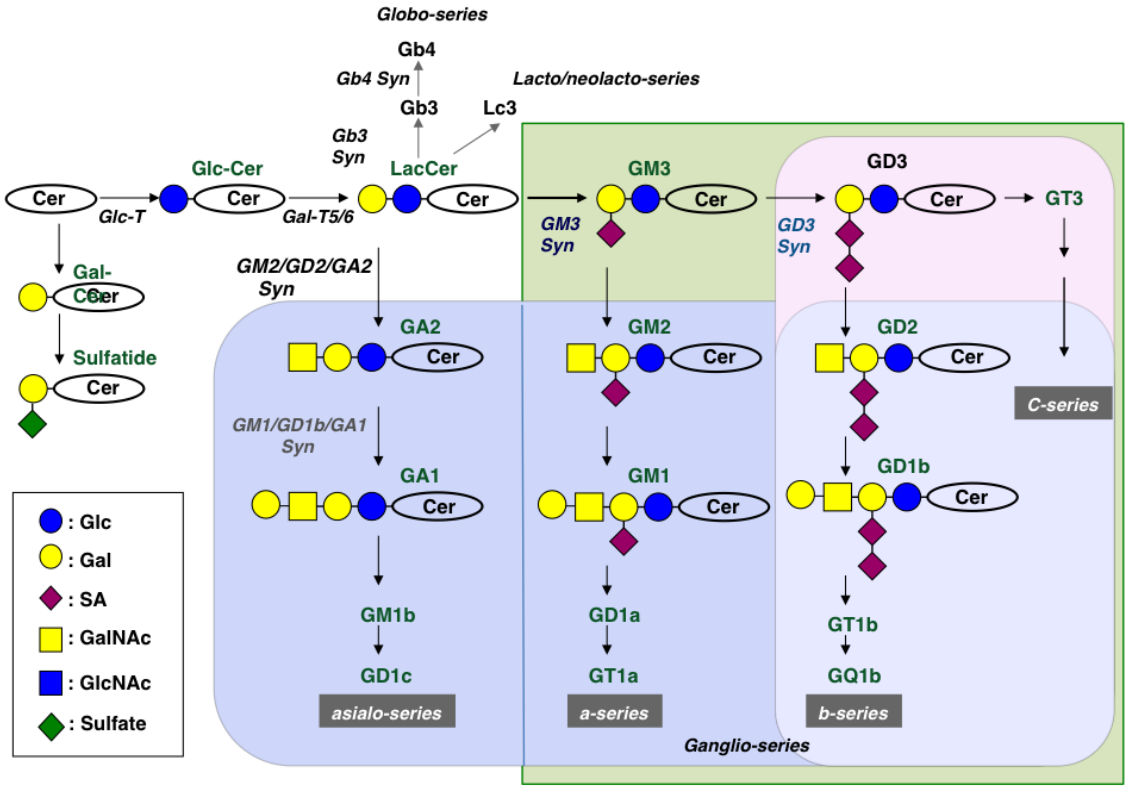

Fig. 2. Main pathway of ganglioside synthesis. Glycosyltransferases catalyzing individual steps were shown by italics, and deleted structures in KO mice were encircled by different colored squires.

On the other hand, ganglioside composition in brain tissues showed almost no change at adult stage, while total amounts gradually reduce. In particular sites in brain,

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however, gradual changes emerge along with aging [9]. Eventually, no comprehensive and fine studies on ganglioside expression in nerve tissues have actually been performed to date aside from exceptions [14,15].

2-2. Function of monosialylgangliosides and disialylgangliosides

In order to analyze regulatory function of nervous systems by gangliosides, a rat pheochromocytoma cell line, PC12 has been frequently used [16,17]. PC12 cells over-expressing GM1 showed reduced sensitivity to nerve growth factor (NGF), leading to lowered neurite extension reaction, and exhibited suppressed activation of TrkA/Ras/ERK1/2 signals upon NGF stimulation [18]. On the other hand, GD3-overexpressing PC12 cells showed increased phosphorylation levels of TrkA and ERK1/2 even without NGF stimulation [19]. Similarly, contrastive effects of gene expression between GM1 synthase and GD3 synthase on phenotypes of various cancer cell lines have been observed [20,21]. From these results, it was demonstrated that monosialylgangliosides and disialylgangliosides play distinct roles in the regulation of malignant properties in cancer cells [22].

Although mechanisms for signal regulation in cancer cells and PC12 cells based on the gangliosides with different numbers of sialic acids are not clear yet, it might be a reflection of regulatory functions of gangliosides in organogenesis and differentiation of nervous tissues and cells. Dynamic changes in ganglioside expression during evolution and development of nervous tissues, and their implication have been summarized in the next chapter.

3. Aging, neurodegeneration and gangliosides

In various tissues and cells, only nervous tissues have been thought to show consistent compositions among species of mammals and birds. In fact, highly similar TLC patterns of gangliosides extracted from brain tissues were reported [23]. However, various changes in ganglioside composition under physiological and pathological conditions have been investigated [2,24,25].

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3.1 Changes of gangliosides in central nervous systems with aging

There have been some reports on the changes of ganglioside expression during evolution, development and aging of mice and rats [26-28]. For human brain gangliosides, changes of ganglioside expression have been reported. Generally, contents of gangliosides gradually decrease [29], and a-series gangliosides tend to decrease mainly in frontal cortex [30]. On the other hand, b-series gangliosides decrease in cerebellum with aging. Whole amounts of lipids contained in brain tissues continuously reduce until reaching 90 years old. In particular, expression levels of gangliosides and cerebrosides largely lowered at that time point. Investigation of 118 individuals with 20 ~ 100 years-old revealed that concentration of gangliosides were maintained between 20 ~ 70 years-old [31], and then, the expression patterns continuously altered with aging, leading to reduced ratios of GM1 and GD1a. Similar findings with these results have been reported based on newest analytical techniques with mass spectrometry [32]. However, there are no detailed researches on the functional relationship of altered ganglioside expression with pathological changes in brain tissues.

3.2 Gangliosides in Alzheimer disease

In a variety of neurodegenerative diseases, particularly in Alzheimer diseases, one of the most important factors involved in neuron death is local inflammation [33]. As reported previously, all components involved in the classic pathway of complement activation were detected in the nervous tissues, and this pathway have been activated in Alzheimer diseases, resulting in the detection as fibrous β amyloid [33] or neurofibrillary tangle [34]. From these findings, complement activation and subsequent inflammation seem to be main mechanisms for the induction of brain damages in Alzheimer diseases.

In these past 20 ~ 30 years, it has been demonstrated that the complement system was involved in the neurodegeneration such as Alzheimer disease [35]. C1q and other components deposit in amyloid plaques and neurofibrillary tangle [36,37] leading to

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complement activation. At the legioned areas, levels of mRNAs for complement components markedly increased [38]. Furthermore, C1q inhibitors alleviated clinical features of Alzheimer diseases, suggesting that complement activation play important roles in the neuroinflammation and subsequent neurodegeneration [39]. Interestingly, the complement activation observed in double knockout (DKO) mice (deleting GM2/GD2 synthase and GD3 synthase) containing only GM3 plays similar roles with those in Alzheimer disease. This point will be described later.

On the other hand, complement systems might work both detrimental and beneficial directions in the disease control [40]. For example, roles during developmental processes [41] or neuronal generation in adults [42] have been reported. In particular, its physiological roles in the elimination of unnecessary cellular components or in the improvement of inflammatory reaction are intriguing.

As a role of gangliosides in Alzheimer disease, GM1 has been reported to become a triggering factor to cause aggregation of Aβ peptide on the cell membrane [43], and mechanisms for the continuous accumulation of Aβ has been also proposed [44]. Murine models for Alzheimer disease generated with genetic back grounds of knockout of GD3 synthase gene or GM2/GD2 synthase gene have been generated, resulting in the milder phenotypes in the former [45], and more serious phenotypes in the latter [46]. Recently, it was reported that expression of *B4GALNT1* promoted the processing of Aβ [47], and complex gangliosides exacerbated clinical features of Alzheimer disease. On the other hand, different changes in ganglioside composition were found in various transgenic model mice expressing human amyloid precursor proteins. [48]. From these results, it has been indicated that altered metabolism of gangliosieds is involved in the pathogenesis of Alzheimer disease.

3.3 Parkinson diseases and gangliosides

It has been reported that amounts of GM1 and GD1a reduced in the brain of patients with Parkinson disease [49]. Furthermore, it was also indicated that *B4galnt1* (GM2 synthase) KO mice lacking GM1 ganglioside [50] showed Parkinson disease-like

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neurological disorders even in heterozygotes [51]. Indeed, efficiency of GM1 administration to Parkinson disease model animals was reported [52], and similar trials have been also performed in clinical cases, resulting in the improvement of clinical features [53]. On the other hand, GD3 synthase（*ST8SIA1*）KO mice lacking b-series gangliosides and having increased levels of GM1 and GD1a [54] were reported to be less susceptible to Parkinson disease [55], suggesting that complementation of GM1 and/or GD1a is also efficient for patients with other neurodegenerative diseases [49].

3.4 Inflammatory reaction and gangliosides

Changes in ganglioside expression in various inflammatory reactions have been observed to date [56]. For example, changes in gangliosides expressed on glial cells [57] or multiple sclerosis, or changes by growth factors [58] have been reported. Further more, it was reported that ganglioside GD3 was induced in inflammatory environments of brain tissues in mice and rats [59]. As for immune cells, GD3/GD2 were induced in T lymphocytes when stimulated via T cell receptor or IL-2 receptor, or various mitogenic factors [60,61]. On the other hand, ganglioside GD2 was expressed on functional T cells [62]. These results corresponded with facts that GD2 was induced on HTLV-1-infected T cells via a viral product p40tax [63]. Thus, changes in ganglioside expression patterns on immune cells are frequently observed in various inflammatory reactions [64,65].

4. Functions of glycolipids elucidated by ganglioside-deficient (knockout) mice

4.1. Abnormal phenotypes exhibited by knockout mice and inflammatory reaction

In order to investigate roles of glycosphingolipids in our bodies, cDNAs of various glycosyltransferase genes have been isolated, i.e. GM2/GD2synthase [66], GD3 synthase [67-69], GM1/GD1b/GA1 synthase [70], GM3 synthase [71-73], Gb3 synthase [74-76] and many other glycosyltransferase cDNA, and then, knockout (KO) mouse lines of these enzyme genes have been established. For example, KO mouse lines of GM2/GD2 synthase [50], GD3 synthase [52], Gb3 synthase [77],

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lactosylceramide (LacCer) synthase [78,79], and double KO mice of GM2/GD2 synthase and GD3 synthase [80,81] have been established and analyzed. DKO mice of *B6galt5* and *B4galt6* were also generated [82]. Synthetic pathway of main glycosphingolipids and glycolipid structures deleted in the individual KO mice are presented in Figure 2. Generally, abnormal phenotypes observed in these KO mouse lines were fairly milder than expected. This could be because residual glycolipids could compensate functions that deleted structures primarily exerted [50]. All these results were summarized in Table 1. However, it seems very intriguing that inflammatory reaction was found mainly in the central nervous systems of many of these KO mice of glycosyltransferase genes [83]. Biochemical and morphological changes observed in the DKO mice of GM2/GD2 synthase and GD3 synthase genes were also detected more or less in many of single gene KO mouse lines [84] (Table 1).

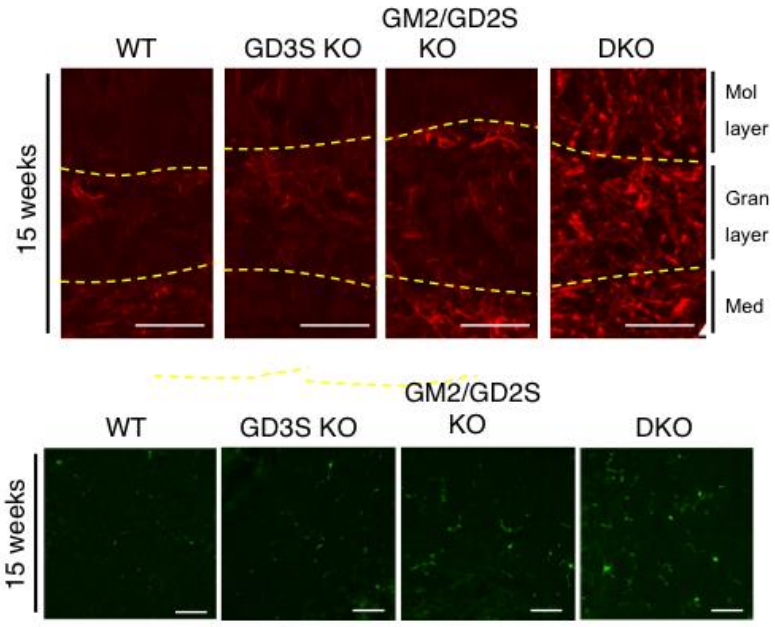

Fig. 3. Immunohistochemistry of GFAP-positive astrocytes and F4/80 antibody-reactive microglia.
Marked gliosis were found in ganglioside synthase gene KO mice. GFAP-positive cells were increased (upper), and F4/80-positive cells (lower) were assembled in cerebella, indicating astrocytes and microglia, respectively.

We reported involvement of complement systems in the neuro-inflammation as a novel aspect of ganglioside deficiency [83]. We compared degree of the complement

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activation, inflammatory reaction, and destruction of lipid rafts among various KO mice of glycosyltransferase genes and wild type (WT) mice, and demonstrated extensively increased expression levels of complement-related genes. More over, we reported proliferation of astrocytes and assembly of microglia corresponding to the degree of the defects in ganglioside composition in the individual KO mice (Figure 3). It was also shown that various cytokine genes were up-regulated with aging correspondingly with the progression of neuro-inflammation as described above. The molecular mechanisms for these inflammation based on the ganglioside deficiency have been analyzed with focus on the changes in lipid rafts [84]. Details were described in the next chapter.

4.2 Neuro-inflammation corresponding to the degree of ganglioside deficiency

Gangliosides have been considered to be involved in the development, differentiation, and function of nervous systems [85]. However, gangliosides have been shown to play roles mainly in the maintenance and repair of nervous tissues based on the abnormal phenotypes detected in the genetically engineered mutant mice [86]. Generally, neurodegeneration was found in common in KO mouse lines of ganglioside synthase genes [50,87,88]. In particular, age-dependent progressive neurodegeneration was observed in KO mice of GM2/GD2 synthase, while subtle abnormal neurological signs could be detected when they were born [50]. On the other hand, DKO mice of GM2/GD2 synthase and GD3 synthase gene demonstrated apparent neurodegeneration in the early stage of life [80], or even sudden death by auditory stimulation [89]. Although KO mice lacking GlcCer synthase [90] showed embryonal lethality [91], conditional KO mice, in which GlcCer synthase was deleted after birth, also showed neurodegeneration [39]. These results indicated that ganglioside deficiency cause abnormality in the maintenance of integrity in the nervous system, leading to neurodegeneration. However, it had been not clear how ganglioside deficiency cause neurodegeneration.

Among various features indicating inflammation in the nervous tissues, abnormal

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proliferation of astrocytes and assembly of microglia were markedly and characteristically found in the cerebella of ganglioside deficient mice [83]. Further more, those inflammatory reactions were confirmed by immunohistochemistry such as GFAP-positive astrocytes and F4/80 antibody-reactive microglia (Figure 3). GFAP+ cells apparently increased at 15 weeks after birth in DKO mice, and further increased with aging. At 50 weeks after birth, GFAP+ cells increased even in the single gene KO mice such as GD3 synthase KO or GM2/GD2 synthase KO. Microglia cells also showed increased assembly at 15 weeks after birth in DKO mice, and further increased with aging. This microglia assembly could be also found at 50 weeks after birth in the single gene KO mice [83].

As for inflammatory cytokines, increased expression levels of IL-1β and TNFα genes were detected in RT-PCR of mRNA from cerebella of DKO mice. Expression levels of these genes tended to increase with aging in DKO mice, while no apparent changes in those gene expression could be found in WT and single gene KO mice.

4.3 Involvement of complement system in the inflammatory reaction

From results of gene expression analysis in DKO mice, it was demonstrated that complement-related genes were generally up-regulated in the cerebella of DKO mice. Therefore, it was suggested that wide range consumption of complement components was induced due to activation of complement system [83]. Actually, deposit of C1q, a complement component, could be found in the cerebella of DKO, and it was also the case in KO mice of GM2/GD2synthase. [83,92]. In order to investigate whether complement activation detected in the cerebella of DKO mice exacerbates with aging, expression levels of C1qa, C3, and C4 were examined along with aging, resulting in the increase of expression of C1q gene between 15 weeks to 50 weeks after birth, and in apparent differences from WT mice with aging. In GM2/GD2 synthase KO mice, expression of complement-related genes moderately increased. Similar inflammatory reactions were also observed in spinal cords of these KO mice [93].

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To clarify the roles of complement activation in the neuro-inflammation and neurodegeneration, triple KO mice lacking complement C3 gene as well as GM2/GD2 synthase and GD3 synthase genes were established as shown in Figure 4 [83]. In this TKO mice, it was really shown that complement activation is actually involved in the complement deposition and secretion of inflammatory cytokines, and also in the neurodegeneration as demonstrated by the alleviation of brain degeneration indicated by reduction in brain weights. In the neurological disorders such as Guillan-Barre syndrome and Miller syndrome caused by anti-ganglioside antibodies, it was reported that complement systems are tightly involved [94,95]. Therefore, inhibitors for complement-related components have been tried in the therapeutic application for the control of these diseases, showing nice effects on mouse disease models [96] and

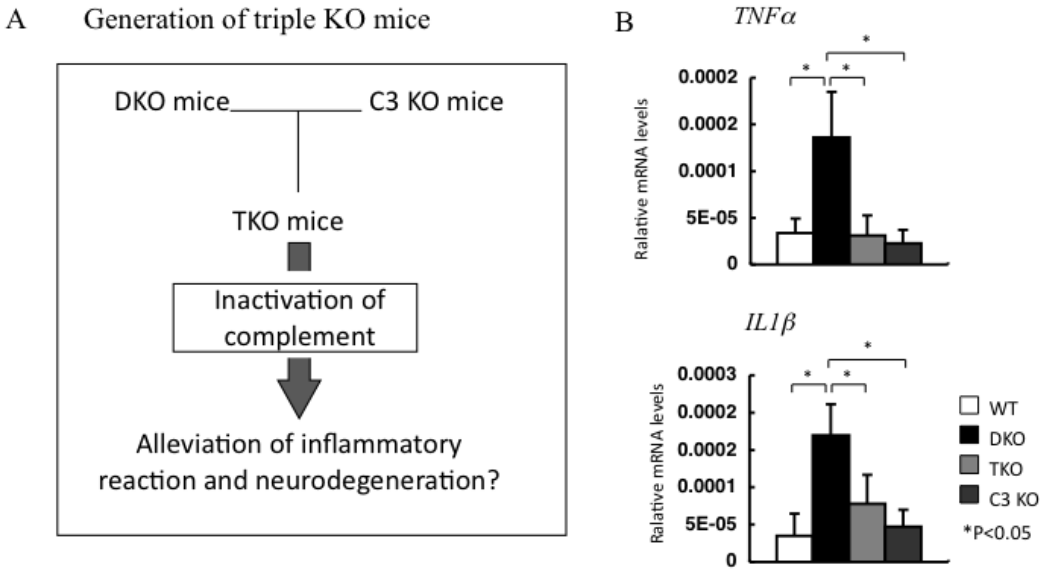

Fig. 4. Inflammatory reactions in the DKO mice were suppressed by genetic disruption of complement system. A, Triple KO (TKO) mice was generated by mating the DKO mice and C3 KO mice to clarify the roles of complement systems in the brain disorders in DKO mice. B, Expression levels of inflammatory cytokines were reduced in TKO mice.

human cases [97].

5. Microdomains on cell membrane and gangliosides

Generally speaking, membrane microdomains such as lipid rafts,

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glycolipid-enriched microdomain (GEM)/rafts, or detergent-insoluble microdomains (DIM) are considered to be a platform of cell signaling, and roles of glycosphingolipids in lipid rafts have been increasingly recognized [98]. In particular, molecular composition of gangliosides consisting of polymorphic sugar chains and heterogenous lipid moiety has made us to expect that gangliosides might be one of main regulators of biological properties of the microdomains.

5.1 Gangliosides regulate cell signaling in microdomains

Various extrinsic stimulations are transduced via receptors and their adjacent molecules on the cell membrane, and these molecules often form molecular complexes in microdomains such as lipid rafts or GEM/rafts [99,100](Figure 5). With a number of experiments using cell lines and KO mice, it has been shown that changes in ganglioside expression largely affect lipid rafts, and control the cell signals and finally cellular phenotypes [98]. Therefore, integrity of lipid rafts has been investigated by analyzing changes in intracellular localization of membrane molecules depending on the conditions of glycosphingolipids and cells [101,102]. Immunocytostaining of these membrane molecules has been an efficient approach for the localization of same membrane microdomains. Further more, it seems extremely important to substantially reveal physical interaction among these molecules on the living cell membrane, and would become a prerequisite to clarify the roles of gangliosides and their associating molecules on the cell membrane [103].

5.2 Microdomain on the cell membrane of nervous systems and gangliosides

Glycosphingolipids are generally considered to be concentrated and localized in GEM/rafts [104], and intense localization is found in highly-differentiated neurons. However, they are dispersed from lipid rafts under particular environments [105 Harder and Simons 1997]. On the other hand, changes in relevant molecules such as caveolin-1 affect intracellular localization of of glycosphingolipids [106]. Furthermore, fine distribution analysis of gangliosides using immunoelectron microscopy revealed that different ganglioside species (e.g. GM1 and GM3) showed distinct distribution

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patterns on the cell membrane, suggesting the presence of heterogeneous microdomains, and individual gangliosides form specific microdomains [107].

Surprisingly, GPI-anchored proteins and GEM/raft markers dispersed from GEM/rafts in cerebella of ganglioside-deficient mice, and marked cell damages were induced [83,84,108]. Analysis of altered floating patterns of GPI-anchored proteins and GEM/rafts markers in various ganglioside-deficient mouse lines by immunoblotting revealed that flotillin-1 and caveolin-1 were apparently dispersed from GEM/rafts (Figure 5). DAF and NCAM showed marked dispersion from GEM/rafts in DKO mice, and DAF shifted to non-GEM/rafts fraction in GM2/GD2 synthase KO mice. Total protein amounts showed no differences among these lines. Generally, gangliosides are essential in the architecture of GEM/rafts, and it is suggested that more striking abnormalities of GEM/rafts were exhibited in DKO mice than in single gene KO mice.

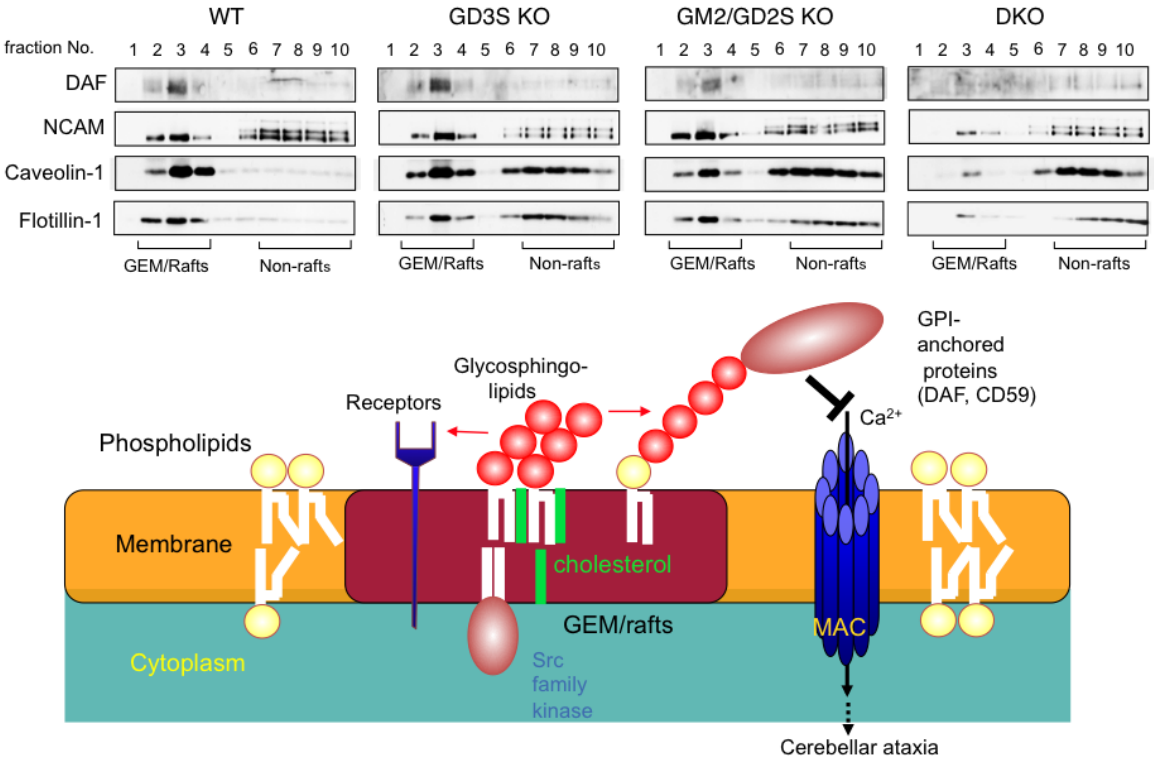

Fig. 5. Destruction of GEM/raft induced changes in the architecture and functions of raft-localizing molecules. Localization of GPI-anchored proteins including complement-regulatory molecules changed, leading to functional abnormalities and subsequent complement activation and inflammation in ganglioside-deficient mice brain.

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5.3 Complement activation and destruction of lipid rafts

Results of analysis of DKO mice revealed that there were many examples of molecular dispersion of GPI-anchored proteins and GEM/raft markers even in single gene KO mice, suggesting that disordered GEM/rafts in brain tissues induce dysfunction of GPI-anchored proteins. In GM2/GD2 synthase gene KO and DKO, localization of GalCer, phospholipids, and cholesterol tended to decrease [84], suggesting that abnormal composition of gangliosides might induce abnormal distribution patterns of GPI-anchored proteins and raft marker proteins, and also of other lipids.

One of the most important factors involved in the complement activation and resulting neurological disorders should be complement-regulatory proteins. In fact, it has been well known that expression levels of CD59 largely lower at the disease site of Alzheimer disease [109]. DAF is also a crucial molecule for the maintenance of tissue integrity [110]. Many of these complement-regulatory factors belong to GPI-anchored proteins, and are concentrated in GEM/rafts fraction [111]. Therefore, it is suggested that destruction of GEM/raft induced changes in the localization of GPI-anchored proteins and their functional abnormalities, leading to complement activation and inflammation. These processes were summarized in Figure 5.

6. Human diseases caused by congenital deficiency of gangliosides

Following analysis of ganglioside functions using KO mice of glycosyltransferase genes, human cases of congenital defects of ganglioside synthase genes were reported in this century.

6.1 GM3 synthase deficiency causes severe clinical features

Although there have been a number of studies on deficiency of ganglioside catalytic enzyme genes, no reports on congenital deficiency of ganglioside synthase genes have been found until 2004. Simpson et al. reported “infantile epilepsy” families in Amish due to deficiency of GM3 synthase (*ST3GAL5*) as the first case of genetic

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mutation in ganglioside synthase genes [112].

As described above, the majority of gangliosides are synthesized through GM3, and diverse carbohydrate structures are generated from a common precursor, lactosylceramide, along with several major synthetic pathways [113]. Therefore, defects of GM3 synthase gene in the Amish families actually resulted in the complete loss of ganglio-series and the patients exhibited serious infantile epileptic disorders [112] and skin abnormalities [114,115], suggesting that gangliosides are essential in the regulation of nervous tissues and other organs. Thus, patients lacking GM3 synthase activity exhibited severe neurological disorders such as infantile epilepsy, mental retardation, visual disorders, and also skin pigmentation, while no definite abnormal phenotypes were found in KO mice of GM3 synthase gene (*St3gal5*) in any sites of the body, except for the auditory system [116].

6-2 GM2/GD2 synthase gene deficiency causes hereditary spastic paraplegia

B4GALNT1 is an essential enzyme for the synthesis of complex gangliosides, lack of which resulted in progressive neurodegeneration with aging in mice [87]. Recently, 11 cases of hereditary spastic paraplegia (HSP) due to mutation in the coding region of *B4GALNT1* were reported [117-119]. We examined the enzyme activities using a cell free enzyme assay with cell extracts, and by flow cytometry of transfectant cells with mutant cDNA expression plasmids [120]. Among them, almost all mutant genes showed complete loss of B4GALNT1 activity, while two mutants showed low activity, indicating that the clinical findings of these patients derived from loss of B4GALNT1 enzyme activity, and the mutations are responsible for the clinical features of HSP. As expected from KO mice phenotypes of *B4galnt1* gene, the intensity of their neurological disorders was milder than expected. These clinical features of the patients including male hypogonadism are very similar with abnormal phenotypes detected in *B4galnt1*-deficient mice [121]. In contrast to GM3 synthase mutation, *B4GALNT1* mutations brought about much milder clinical features with slower progression.

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KO gene	Glc-Cer Syn	GM3 Syn	GD3 Syn	GM2/GD2 Syn	DKO[1)]
Lost structures	all glycosphingo-lipids	ganglio-series (a-, b-, c-)	b-series (and c-series)	all comlex gangliosides (inc. asialo-series)	all comlex gangliosides (inc. asialo-series)
Remaining structures		asialo-series	a-series and asialo-series	GM3, GD3 (and GT3)	GM3
	Gal-Cer and sulfatides	Gal-Cer and sulfatides neutral glycolipids	Gal-Cer and sulfatides neutral glycolipids	Gal-Cer and sulfatides neutral glycolipids	Gal-Cer and sulfatides neutral glycolipids
Phenotypes	↓ Embryonal lethal	↓ No apparent abnormalities	↓ Mild abnormalities	↓ Gradual abnormalities	↓ Neurodegeneration from early phase
Remarks		Auditory disorder	Poor repairment Low serum leptin	Male infertility Low serum testostelone Wallerian degen[2)]	Refractory skin lesion Auditory shock

Table 1. Deficient structures, remaining glycolipids and phenotypes of individual KO mice of glycosyltransferase genes. Residual glycolipids could compensate functions that deleted structures primarily exerted. [1)], double KO of GD3 synthase and GM2/GD2 synthase genes. [2)], Wallerian degeneration.

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7. Future scope of ganglioside research

Recent advances in methodologies and technologies have enabled us to further investigate modes of action for gangliosides. Fine heterogeneity in either sugar moiety or ceramide portion has been demonstrated, leading to further understanding of mechanisms by which gangliosides play their roles or interact with their recognizing molecules. For example, further analysis of derivatives of sialic acids such as deaminoneuraminic acid (KDN) [122], *O*-acetylated GD3/GD2 [123] as sugar modifications, and long fatty chain-containing glycolipids in lactosylceramide [124], saturated/unsaturated fatty acid-containing Gb4 [125], or hydroxylated ceramide-containing gangliosides [126]. remain to be promoted. It is also important issue to be solved which cell lineages more critically need gangliosides between neuron and glia [127], although few studies have been reported to date. Ultra-high-resolution imaging of gangliosides in GEM/rafts has enabled us to understand actual formation of microdomains [128] or new concept of gradual formation of GEM/rafts with different size and composition [129]. Identification of novel ligand molecules for gangliosides should be also promoting factor for further understanding of molecular functions of gangliosides [103, 130], although not so many studies in neurology field have yet been reported to date.

8. Conclusion

It seems hard now to clearly answer the fundamental question, what are roles of gangliosides in nerve functions. Many of “functions” presented here are results drawn from observation of abnormal situations brought about by artificially enhanced expression of particular glycosyltransferases or suppression of their functions in turn to speculate normal functions primarily exerted. Therefore, they represent a part of “functions”, but not all. The main factor to make us feel unsatisfactory is the technical restriction in the manipulation of key glycosyltransferase genes, observing some phenotypes as functions of many structures belonging to one group all together. Never the less, clarification of roles of individual gangliosides by collecting experimental

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data with various limitations should be essential for the solution of mystery in the polymorphism of carbohydrates, leading to the new pavement toward our ultimate purpose.

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Figure legends

Fig. 1. Glycosphingolipids are amphipathic molecules, and expressed in the out layer of lipid two layers of cell membrane. Hydrophobic portion (ceramide) is embedded in the outer layer of the membrane, and sugar portion is protruding outside of the membrane.

Fig. 2. Main pathway of ganglioside synthesis. Glycosyltransferases catalyzing individual steps were shown by italics, and deleted structures in KO mice were encircled by different colored squires.

Fig. 3. Immunohistochemistry of GFAP-positive astrocytes and F4/80 antibody-reactive microglia.

Marked gliosis were found in ganglioside synthase gene KO mice. GFAP-positive cells were increased (left), and F4/80-positive cells (right) were assembled in cerebella, indicating astrocytes and microglia, respectively.

Fig. 4. Inflammatory reactions in the DKO mice were suppressed by genetic disruption of complement system. A, Triple KO (TKO) mice was generated by mating the DKO mice and C3 KO mice to clarify the roles of complement systems in the brain disorders in DKO mice. B, Expression levels of inflammatory cytokines were reduced in TKO mice.

Fig. 5. Destruction of GEM/raft induced changes in the architecture and functions of raft-localizing molecules. Localization of GPI-anchored proteins including complement-regulatory molecules changed, leading to functional abnormalities and subsequent complement activation and inflammation in ganglioside-deficient mice brain.

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Table 1. Deficient structures, remaining glycolipids and phenotypes of individual KO mice of glycosyltransferase genes. (attached in figures)