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MINI-REVIEW

GM1 ganglioside and Alzheimer's disease

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Abstract Assembly and deposition of amyloid ß-protein (Aß) is an invariable and fundamental event in the pathological process of Alzheimer's disease (AD). To decipher the AD pathogenesis and also to develop disease-modifying drugs for AD, clarification of the molecular mechanism underlying the Aß assembly into amyloid fibrils in the brain has been a crucial issue. GM1-ganglioside-bound Aß (GAß), with unique molecular characteristics such as having an altered conformation and the capability to accelerate Aß assembly, was discovered in an autopsied brain showing early pathological changes of AD in 1995. On the basis of these findings, it was hypothesized that GAß is an endogenous seed for amyloid fibril formation in the AD brain. A body of evidence that supports this GAß hypothesis has been growing over this past 20 years. In this article, seminal GAß studies that have been carried out to date, including recent ones using unique animal models, are reviewed.

Keywords Alzheimer's disease · Amyloid ß-protein · Ganglioside · GM1-ganglioside-bound Aß (GAß) · Seed · Conformational transition

Introduction

The assembly and deposition of physiologically generated, soluble proteins such as the amyloid ß-protein (Aß) in organs such as the brain is a critical step in the pathological process of various human amyloidoses, including Alzheimer's disease (AD). In AD, the level of soluble Aß may somehow be increased, which leads to its assembly into amyloid fibrils in the brain. In most but not all familial AD cases, the expression of responsible genes enhances Aß production. However, it remains unclear whether an increase in Aß production by the expressed pathogenic genes is sufficient to cause spontaneous Aß assembly in the brain. Moreover, there has been no evidence of increased Aß production or impaired Aß clearance in sporadic AD, the principal form of the disease. More importantly, an increase in the level of soluble Aß, if any, cannot explain why there is preferential assembly and deposition of Aß in certain brain regions, *e.g.*, the precuneus, but not in other regions, *e.g.*, the calcarine cortex. Yanagisawa *et al.* discovered GM1-ganglioside-bound Aß (GAß), which has unique molecular characteristics such as having an altered conformation and the capability to accelerate Aß assembly, in an autopsied brain showing only early pathological changes of AD [1]. On the basis of these molecular characteristics of GAß, Yanagisawa *et al.* hypothesized that GAß is an endogenous seed for amyloid fibril formation in the AD brain [1, 2]. To date, various *in vitro* and *in vivo* studies have provided evidence that supports the GAß hypothesis [for review see 3–5]. Recent studies using animal models also shed new light on the pathological significance of GAß [6–8].

Conformational transition of Aß through interaction with gangliosides

Clarification of the supermolecular process of conformational transition of soluble Aß through interaction with gangliosides should be a fundamental issue to understand the pathological significance of gangliosides in Aß assembly. It was suggested by previous studies that Aß adopts an α-helix structure through its binding to gangliosides [9–11]. This line of evidence is in very good agreement with our knowledge on the

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misfolding-type amyloidogenesis, in which a given amyloidogenic protein, usually showing a unordered structure, adopts an α-helix structure prior to its assembly into fibrils with the ß-sheet structure [12] (Fig. 1). Kato and his colleagues conducted further studies regarding this issue using circular dichroism (CD) and nuclear magnetic resonance (NMR) techniques [13, 14]. They successfully demonstrated that first, two α-helical regions are formed in Aß through the interaction of Aß with lyso-GM1 ganglioside micelles, and second, this conformational transition needs the inner part of a ganglioside but not the outer carbohydrate branches, which are required by microbial toxin and viruses.

Aß assembly on GM1-containing membranes

The next question is how the initial binding of soluble Aß to GM1 ganglioside triggers the subsequent Aß assembly to form amyloid fibrils with the ß-sheet structure. Apparently, monitoring the conformational transition of a single, soluble Aß molecule is extremely difficult with currently available techniques. However, according to the GAß hypothesis, a possible scenario would be as follows. Once GAß is formed on neuronal membranes, another soluble Aß binds to GAß and adopts a similar conformation as GAß, and then serves as a new seed or template for subsequent binding and conformational transition of the next Aß, subsequently resulting in amyloid fibril formation. The point of this scenario is the sharing of a unique conformation by GAß and the Aß at the ends of growing fibrils. This possibility has been indicated by a finding that a monoclonal antibody raised against purified GAß from human brains specifically recognizes the Aß at the ends of growing fibrils [2]. Alternatively, it was also suggested by Kakio *et al.* [10] that soluble Aß initially adopts an α-helix structure through its binding to GM1 ganglioside on membranes, and then transforms into a ß-sheet structure as the Aß density on the membrane increases. The possibility of interaction between Aß molecules following their binding to GM1 gangliosides has been supported by the finding of the

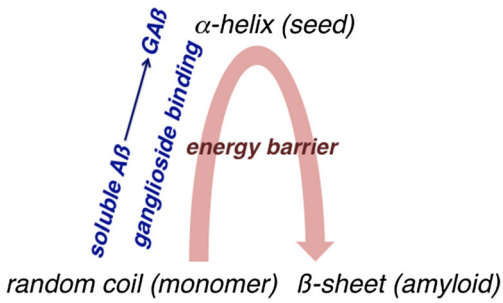

Fig. 1 Conformational transition of Aß from random coil to α-helix through binding to ganglioside. In most cases of misfolding-type amyloidosis, an given amyloidogenic protein such as Aß adopts α-helix before further transforming into ß-sheet-rich amyloid

same group [15, 16] and another group [17]. Another group also suggested that the initial conformational change of Aß to α-helix on the GM1ganglioside-containing membrane ends with dimerization adopting the ß-strand structure, which potently leads to further development of aggregates with higher-ordered structures [18].

Prerequisite of GM1 ganglioside clustering to induce GAß generation

Given that Aß is physiologically secreted into the extracellular space and GM1 ganglioside is also physiologically expressed on the surface of cells, it has been an enigma why GAß is generated only in the brains that are destined to develop amyloid deposition. In regard to this issue, Matsuzaki and his colleagues carefully examined how Aß binds to GM1 ganglioside on the membrane surface using liposomes with various lipid compositions. Notably, they found that first, GM1 ganglioside clustering is prerequisite for the Aß binding, and second, cholesterol in the membranes markedly facilitates the GM1 ganglioside clustering [19]. Thereafter, a study using cultured cells suggested that sphingomyelin (SM) also induces GM1 ganglioside clustering, leading to GAß generation [20]. Hoshino and his colleagues have recently confirmed using computer simulation techniques that the lipid environment is indeed a crucial factor for GM1 ganglioside clustering [21]. They performed molecular dynamics simulation on two types of membrane composed of GM1/SM/cholesterol and GM/POPC and showed that first, GM1 ganglioside condensation occurs only in GM1/SM/cholesterol, and second, in the GM/SM/cholesterol bilayer, lipids interact with each other through hydrogen bonding in their head groups. The role of hydrogen bonding between lipids in providing the favorable milieu for the Aß binding to GM1 ganglioside has been also clarified by Fantini *et al.* [22]. Furthermore, the critical requirement of GM1 ganglioside clustering for induction of GAß generation has also been suggested by atomic force microscopy study conducted by Matsubara *et al.* using lipids extracted from synaptosomes from an aged mouse brain [23]. On the basis of the studies that have been conducted to date, cholesterol in surrounding lipid environments is likely the strongest driving force that induces GM1 ganglioside clustering. In this context, noteworthy is the results of a recent study of human brains obtained from aged individuals of different *APOE* genotypes by Oikawa *et al.* [24]. They analyzed the lipid composition of the synaptic plasma membrane (SPM) isolated from the cerebral cortices by liquid chromatography-mass spectrometry. Interestingly, *APOE* ε2, which potently inhibits amyloid deposition, significantly decreased level of cholesterol in SPM. Given that the efficiency of cholesterol efflux from the surface of neuronal membranes is likely regulated by the *APOE* genotype, apoE2, which is encoded by *APOE* ε2, may induce

less accumulation of cholesterol in SPM. Apart from such a direct effect on cholesterol level in neuronal membranes of the *APOE* genotype, it is largely unknown whether risk factors for AD or cell pathology preceding amyloid deposition development cause alterations in the lipid composition of neuronal membranes. Yuyama *et al.* focused on one of the cell pathological features of neurons, endosomal-lysosomal impairment [25], in the AD brain. Yuyama *et al.* examined whether induction of endosomal-lysosomal impairment causes alteration in the lipid composition of neuronal membranes and induces GAß-dependent amyloid deposition. Notably, they found that experimental endocytic disorder of cultured neurons potently induced GM1 ganglioside accumulation, which led to GAß-dependent amyloid deposition at presynaptic neuritic terminals [26, 27]. Facilitation of GAß generation by endosomal-lysosomal disorder has also been suggested by a study using a mouse model of human lysosomal dysfunction diseases [28]. Very recently, it has been reported that amyloid deposition is facilitated in association with enhanced GAß generation in a nonhuman primate model of diabetes mellitus (DM) [8]. Interestingly, in this model of DM, the expression levels of small GTPase proteins, including Rab5 and Rab7, which are involved in the regulation of the membrane traffic of the endosomal-lysosomal system, were significantly increased. Given that DM is one of the strong risk factors for AD development, this model may shed new light on the molecular mechanism by which amyloid deposition is facilitated through GAß generation. Thus, taken all together, it is likely that various risk factors for AD development induce alteration in the distribution and/or composition of neuronal membrane lipids, leading to formation of GM1 ganglioside clustering, and then GAß generation (Fig. 2). In addition, alterations in ganglioside

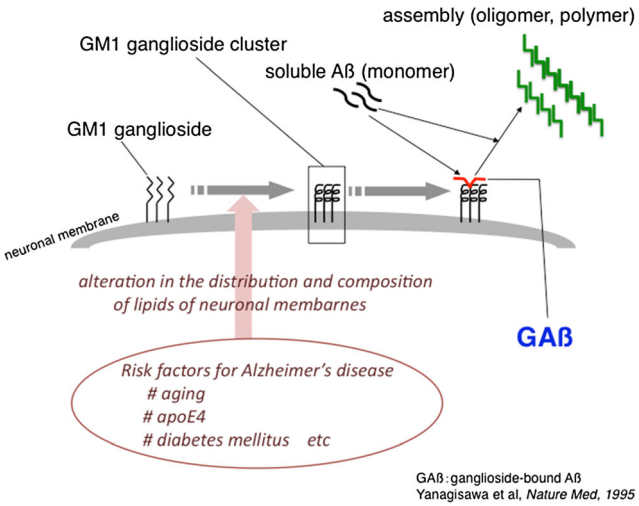

Fig. 2 Hypothetical scheme of GAß generation. Various risk factors for AD development, including aging, expression of apoE4 and diabetes mellitus, induce alteration in the distribution and/or composition of neuronal membrane lipids, leading to formation of GM1 ganglioside cluster, and then GAß that acts as a seed for amyloid fibril formation in the brain

structure may also be an alternative driving force to induce GAß generation as recently reported by Oikawa *et al.* [29]. They performed a study on SPMs isolated from human brains, including amyloid-bearing precuneus, and found that imbalance in fatty-acid-chain length of gangliosides is responsible for GAß-dependent Aß assembly in the precuneus.

Implication of GAß as a possible diagnostic and therapeutic targets for AD

Recently, GAß has been detected at substantial levels in an *hAPP* transgenic mouse model as young as 3 months, at which age amyloid deposition has not developed yet [7]. This finding is in excellent agreement with the discovery of GAß in the human brains with early but not advanced pathological changes of AD [1]. Furthermore, GAß has also been detected in human cerebrospinal fluid (CSF) with levels that well correlated with those of Aß1-42, a major constituent of amyloid deposited in the human brain. All these findings imply that monitoring GAß in the brain or in CSF may be beneficial in the early detection of AD pathology before the onset of clinical signs of dementia or even prior to the emergence of major pathological changes of AD, including amyloid and tau pathologies, inflammation and/or neuronal loss. In addition, it has recently been shown that administration of a small compound of ß-hexosaminidase activity enhancer potently corrects the behavioral phenotype of AD-related transgenic mouse model in association with GAß level reduction [6]. The precise mechanism underlying GAß level reduction by ß-hexosaminidase activity enhancement remains to be clarified; however, this pioneering study provides a possibility that mild modulation of ganglioside metabolism, which could be safely performed without inducing serious adverse effects, may be clinically beneficial as a preemptive therapy of AD.

Conclusions

The seeded polymerization theory was widely accepted to explain the molecular mechanism underlying fibril formation from amyloidogenic proteins such as Aß [30, 31]. However, in the case of Aß, an extremely high level is required for the spontaneous nucleation to form seeds for subsequent amyloid fibril formation. On the basis of the kinetics of Aß nucleation, Esler *et al.* reported that formation of Aß seeds would take at least thousands of years to occur spontaneously at physiological Aß levels [32]. Thus, it is questionable whether Aß nucleation spontaneously occurs under biological conditions, even in the case of expression of genes responsible for familial AD, which may enhance Aß production tenfold at the highest. Taking also into account the region specificity of amyloid deposition in the brain, it is reasonable to assume the existence

of some "facilitator(s)" for the initiation of Aß assembly. To the best of our knowledge, GM1 ganglioside is the only molecule confirmed to function as a facilitator of Aß assembly. In addition, note that amyloid fibrils formed in the presence of GM1 ganglioside show marked neurotoxicity [33, 34]. Thus, Aß assembly into amyloid fibrils induced by gangliosides should be an important issue to decipher the molecular mechanism underlying amyloid emergence in AD and to develop diagnostic and therapeutic strategies for AD. Also, therapeutic applications of antibodies or small compounds, that specifically recognize the unique structure of GAß, in animal models designed to develop amyloid deposition could be very useful to validate the GAß hypothesis.

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Conflict of interest The author declare that he is free from conflict of interest.

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