Aβ–ganglioside interactions in the pathogenesis of Alzheimer's disease

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Abbreviations: Aβ, amyloid β-protein; AD, Alzheimer's disease; AFM, atomic force

microscopy; APP, amyloid precursor protein; CD, circular dichroism; DAC-Aβ, diethylaminocoumarin-3-carbonyl Aβ; FCS, fluorescence correlation spectroscopy; FTIR, Fourier-transform infrared; GAβ, GM1-bound form of Aβ; PC, phosphatidylcholine; SA, sialic acid; ssNMR, solid state NMR; TEM, transmission electron microscopy.

Abstract

It is widely accepted that the abnormal self-association of amyloid β-protein (Aβ) is central to the pathogenesis of Alzheimer's disease, the most common form of dementia. Accumulating evidence, both in vivo and in vitro, suggests that the binding of Aβ to gangliosides, especially monosialoganglioside GM1, plays an important role in the aggregation of Aβ. This review summarizes the molecular details of the binding of Aβ to ganglioside-containing membranes and subsequent structural changes, as revealed by liposomal and cellular studies. Furthermore, mechanisms of cytotoxicity by aggregated Aβ are also discussed.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia and more than 50 million people worldwide suffer with the disease. AD is classified into the early onset, familial (hereditary) type and the late onset, sporadic type, the major form. It is widely accepted that amyloid β-protein (Aβ), typically composed of 39–43 amino acids residues but most commonly 40 or 42, is central to the pathogenesis of AD (Fig. 1A) [1-3]. First, a pathological hallmark of AD is the cerebral cortical deposition of senile plaques, with a major component being fibrillar Aβ. Second, mutations in familial AD were found either within the Aβ sequence or close to the cleavage sites of single membrane-spanning amyloid precursor protein (APP), from which Aβ is generated by β- and γ-secretases (Fig. 1A). Third, aggregated forms of Aβ rich in β-sheet structures (oligomers and fibrils) impair neuronal cells [4]. Thus, it is of marked importance to elucidate mechanisms whereby soluble Aβ self-assembles into aggregates.

Accumulating evidence has suggested that the binding of Aβ to membranes plays

an important role in the aggregation of Aβ [5-13] as well as other amyloidogenic proteins [14, 15]. Aβ has been suggested to mainly originate from the neuronal cell surface APP, which is subsequently processed in recycling endosomes [3], and is most likely released into the synaptic terminal [16]. Human brains are rich in gangliosides (sugar lipids), the major species of which are GM1, GD1a, GD1b, and GT1b (Fig. 1B) [17]. Yanagisawa et al. identified a specific form of Aβ bound to GM1 in brains exhibiting the early pathological changes associated with AD, and also suggested that the GM1-bound form of Aβ (GAβ) may serve as a seed for the formation of Aβ aggregates [18]. Gangliosides are considered to be localized in detergent-resistant membrane microdomains or lipid rafts with SM, cholesterol, and other lipids with saturated acyl chains [19], although the presence of lipid rafts in living cells is still controversial [20]. Thus, the GAβ-dependent fibrillization is also affected by these raft lipids. The production [21-23] and degradation [24] of Aβ occurs, at least partly, in lipid rafts. Substantial amounts of detergent-insoluble Aβ were detected in lipid rafts in mature rat brains [25], human neuroblastoma cells [26], and the brain of the Tg2576 transgenic mouse [27].

The ganglioside theory can explain both risk factors of AD and region-specific deposition of amyloids in brains. The two major risk factors are aging and the apoE4 allele, with the latter being related to cholesterol metabolism. The GM1 level in synaptosomes increased in old mouse brains [28], and that in detergent-resistant membrane microdomains of synaptosomes increased in age- and apoE4-dependent fashions, and fibrillization by Aβ-(1–40) was enhanced in the presence of synaptosomes from the aged apoE4-knock-in mouse brain [29]. Both GM1 and sphingomyelin were accumulated in synaptosomes of mouse brains in an age-dependent fashion. Synaptosomes from aged mouse brains facilitated fibril formation by Aβ-(1–40), which was attenuated by cholesterol-removing methyl-β-cyclodextrin treatment [30].

Amyloids are deposited region-selectively in brains. The precuneus and calcarine cortex are two of the most vulnerable and resistant regions to amyloid deposition, respectively. Lipids extracted from synaptic plasma membranes isolated from the amyloid-bearing precuneus accelerated aggregation of Aβ-(1–42), whereas this did not happen in the case of the amyloid-free precuneus or calcarine cortex. The enhanced amyloidogenesis was due to an increase in the C20:0 to C18:0 fatty acid ratio of GD1b

[31]. The assembly of the Dutch- (E22Q) and Italian- (E22K) variants of Aβ-(1–40) was accelerated by GM3, which is expressed in the cerebral vessel wall, the site of their deposition in vivo. On the other hand, the fibrillization by the Flemish-variant (A21G) was facilitated by GD3, which is upregulated in the co-culture of endothelial cells and astrocytes forming the cerebrovascular basement membrane, the site of Flemish-variant deposition [32]. The Arctic-mutant (E22G) as well as wild-type, but not the Dutch-variant, rapidly formed fibrils in the presence of GM1, suggesting that GM1 plays a critical role in amyloid deposition in the brain parenchyma [33]. In addition to these observations, various in vivo and cellular studies supported the GAβ hypothesis in more straightforward fashions, as summarized below. Also, refer to other reviews for the pathological roles of ganglioside metabolism in AD [34, 35]. The involvement of gangliosides in amyloidogenesis has been suggested also for other proteins, such as α-synuclein [36] and human islet amyloid polypeptide [37].

2. Involvement of GAβ in the pathogenesis of AD

2.1. In vivo studies

Several *in vivo* studies support the GAβ hypothesis. The antibody 4396C specific for GAβ stained brains with AD and Aβ- overexpressing Down's syndrome [38]. In addition, the administration of an Fab fragment of 4396C chemically linked to a cell-penetrating peptide significantly reduced plaque formation in AD model mice [39]. GAβ accumulation exclusively occurred in early, late, and recycling endosomes before the deposition of senile plaques in an age-dependent fashion in cerebral cortices of cynomolgus monkeys [40]. High levels of GM1-bound Aβ-(1–42) were recovered from brain membrane fractions of human APP transgenic mice. GAβ was also detected in human CSF [41]. Furthermore, a recent imaging mass spectroscopy study revealed that GM1 was present in the core region of plaques in transgenic mice ($tgAPP_{ArcSwe}$) [42], overexpressing the Arctic mutant (E22G) of Aβ, the fibrillization of which is much effectively accelerated by GM1 than the wild-type [32]. A good correlation was found between the amounts of Aβ-$(1–40)_{Arc}$ and GM1 [42]. A significant increase in GM1 and cholesterol was reported in Aβ-positive nerve terminals

from the AD cortex [43]. GM1 and GM2 levels in detergent-resistant membrane microdomains from frontal and temporal cortices of AD brains were increased compared with age-matched controls [44]. It should be noted that *decreases* in the amounts of gangliosides per tissue weight for AD brains were the result of a marked loss of nerve endings [17, 45].

2.2. Cellular studies

Cellular studies also revealed the importance of GM1 in the amyloidogenesis by Aβ. Time-dependent accumulation of fluorescein-labeled Aβ-(1–40) on rat pheochromocytoma PC12 cells and resultant cell death were visualized by confocal microscopy. The protein was colocalized with GM1, more precisely fucosyl GM1 in this cell line [46], as visualized with fluorescently labeled-choleratoxin subunit B (CTX-B). Cholesterol depletion inhibited the deposition of the protein [47]. Amyloid formation by unlabeled Aβ-(1–42) on nerve growth factor-differentiated PC12 was detected by the amyloid-specific dye Congo red. Amyloids were selectively

formed in GM1-rich, cholesterol-rich regions on cell membranes in a time-dependent fashion, and induced cytotoxicity. Inhibition of cholesterol synthesis rescued cells from amyloid formation and cytotoxicity. Interestingly, contents of not only cholesterol but also GM1 were reduced by this treatment, suggesting a link between the biosynthesis of the two lipids [48].

GM1-mediated amyloid formation and neurotoxicity were also observed in neurons. Aβ-(1–40) formed amyloids on primary neurons as well as NGF-treated PC12 cells, inducing shrinkage and retraction of neuritis. The initial deposition occurred at synaptophysin-positive presynaptic sites and the junction between cell bodies and neuritis, respectively. The amyloidogenesis was inhibited by GM1-binding CTXB, anti-GAβ antibody 4396C, or ganglioside synthesis inhibitor treatment [49]. Treatment with GM1-diminishing D-threo1-phenyl-2-decanoylamino-3-morpholino-1-propanol reduced the accumulation of fluorescently labeled Aβ-(1–42) onto hippocampal neurons. Synaptotoxicity by Aβ was ameliorated by CTXB [50]. Treatment with the sphingomyelinase inhibitor GW4869 significantly enhanced SM expression on the PC12 cell surface and facilitated

fibrillization by Aβ-(1–40), although the effects of the compound on the expression level of GM1 were not examined [51].

Endocytic pathway abnormalities precede the deposition of Aβ in sporadic AD [52]. Treatment of PC12 cells with chloroquine, which perturbs membrane trafficking from endosomes to lysosomes, induced the accumulation of GM1 in early endosomes and on the cell surface, inducing amyloid formation [53]. Blocking the late endocytic pathway by Rab7 supression, which increased levels of SM as well as GM1 [51], in NGF-treated PC12 cells induced the enlargement of early endosomes and amyloid formation by Aβ-(1–40), which was blocked by 4396C [54]. Extracellular amyloid formation can be induced by GM1 associated with exosomes [55].

Species differences in susceptibility to AD also support the hypothesis that GM1-containing membranes are major platforms for amyloidogenesis [56]. Aged rodents rarely develop cerebral Aβ amyloid deposition. Rodent Aβ-(1–40) has only the three mutations R5G, Y10F, and H13R. It forms similar water fibrils to the human counterpart. In contrast, on neuronal membranes and GM1-containing raft-like membranes, human Aβ forms toxic amyloid fibrils, whereas rodent Aβ produces less

toxic protofibrils that are not stained by the amyloid-specific dye Congo red.

3. Molecular details of Aβ–ganglioside interactions

This section focuses on Aβ–ganglioside interactions. For more general Aβ–lipid interactions, refer to previous reviews [5, 14].

3.1. Early studies

The interaction of Aβ with various ganglioside micelles was initially investigated. McLaurin and Chakrabartty reported that Aβ-(1–40) and Aβ-(1–42) bind to GM1 micelles, forming mixed α-helix/β-sheet structures and the sialic acid (SA) or *N*-acetylneuraminic acid group is essential for this interaction.[57]. Surewicz's group revealed that Aβ-(1–40) but not Aβ-(1–28) binds to GM1 micelles as well as the pentasaccharide moiety of GM1 [58]. They also reported that the dissociation constants (K_d) of F10W-Aβ-(1–40) were in the order GD1a = GT1b < GM1 < GM2

(Table 1). The value for GM1 was several-fold lower than that for the pentasaccharide moiety of GM1 [59].

Subsequent studies investigated the interaction of Aβ with ganglioside–phosphatidylcholine (PC) mixtures. McLaurin et al. [60] and our group [61] used circular dichroism (CD) spectroscopy and observed that Aβ-(1–40) did not associate with GM1 in egg yolk PC vesicles until the GM1 content exceeded 30%, whereas F10W-Aβ-(1–40) was reported to bind to palmitoyloleoyl PC vesicles containing 3 mol% GM1 added to preformed vesicles [59]. Gangliosides incorporated into PC bilayers facilitate fibrillization of Aβ-(1–40) [59] and Aβ-(1–42) [60], although gangliosides were reported to have no effect on the fibrillization of Aβ-(1–40) [62]. Valdes-Gonzales et al. determined the K_d values of Aβ-(1–42) for ganglioside-embeded dimyristoyl PC monolayers by surface plasmon resonance (Table 1) [63].

3.2. Ganglioside cluster as a binding site of Aβ

We systematically investigated Aβ–ganglioside interactions using carefully

prepared monomeric Aβ, because the initial states of Aβ in solution, i.e., completely monomeric or already partially aggregated, may significantly affect the protein–membrane interactions and different initial states may be a reason why the findings obtained by various research groups often show large discrepancies. For binding experiments we used DAC-Aβ, in which the N-terminus of Aβ was labeled with the diethylaminocoumarin dye. A change in polarity upon membrane-binding induces a significant blue shift and an enhancement in intensity. None of the major membrane lipids including zwitterionic PC, sphingomyelin (SM), anionic phosphatidylserine (PS), and phosphatidylglycerol exhibited detectable binding to Aβ-(1–40) or Aβ-(1–42) at a physiological pH. In contrast, Aβ bound to negatively charged gangliosides, including GM1 [64-67]. The K_d values of DAC-Aβ-(1–40) for various GM1-containing raft-like membranes at 37°C were 0.12–0.15 μM, which are almost identical to the value for unlabeled Aβ-(1–40), suggesting that the DAC moiety does not affect the binding behavior (Table 1). GM1 micelles exhibited a similar binding behavior to GM1-containing bilayers [65], whereas DAC-Aβ-(1–40) binds to GM1 nanodiscs two orders of magnitude more strongly (Table 1) [68].

Interestingly, DAC-Aβ-(1–40) binds to membranes composed of GM1/SM/cholesterol (2:4:4) but not GM1/PC (2:8) in spite of the same GM1 contents (Fig. 2A) [64]. Similarly, fibril formation by unlabeled Aβ-(1–40) occurs in the presence of the GM1/SM/cholesterol mixture, not the GM1/PC liposomes (Fig. 2B) [69]. The former composition resembles that of the lipid raft. Molecular dynamic (MD) simulations [70] as well as excimer fluorescence experiments [64] suggest that the sugar moiety of GM1 forms a string-like cluster in the Aβ-binding raft bilayers, whereas it is uniformly distributed in the Aβ-nonbinding GM1/PC membranes (Fig. 2C) The formation of the GM1 cluster is cholesterol-dependent [64]. Differential scanning calorimetry experiments revealed that in ternary GM1/SM/cholesterol mixtures, GM1- and cholesterol-enriched phases are formed [71]. MD simulations suggested that cholesterol forms many hydrogen bonds with SM and more frequently with GM1, and plays important roles in promoting the assembly of other lipids [70]. This cholesterol dependence is in accordance with the above-mentioned observations that cholesterol depletion attenuates the self-assembly of Aβ [30, 47, 48].

Apparently contrary to the fibrillization-catalyzing activity of GM1 clusters

mentioned above, Amaro et al. reported that GM1 inhibits Aβ oligomerization induced by SM [72]. This study using giant unilamellar vesicles was carried out at a low ionic strength and 26°C. First, electrostatic repulsion between negatively charged Aβ and anionic GM1 are much stronger than that at a physiological ionic strength, inhibiting Aβ–GM1 interaction. Second, sphingolipids are in the gel state at this temperature. Aβ is known to interact with PC in the gel state, but not in the liquid-crystalline state [73].

3.3. Mechanism of Aβ binding to ganglioside clusters

It is not fully understood why Aβ specifically binds to anionic ganglioside clusters, even though the protein is also negatively charged at a neutral pH [67]. The hydrophobic interaction between the C-terminal hydrophobic residues and hydrocarbon core of the bilayer is minimal, as exemplified by the observations that : 1) Aβ only weakly interacts with zwitterionic lipids [74], and 2) the 500-fold difference in the binding constant to GM1 cluster-containing membranes between DAC-Aβ-(1–40) and

DAC-Aβ-(1–28) can be explained by a free energy gain upon a coil-to-helix transition [67]. An MD simulation study suggested that a cluster of SA holds the Lys^{28} side chain of Aβ-(1–42), making its C-terminal region exposed to water in GM1/cholesterol/SM (1:2:2) [75]. Raman spectroscopy suggested that Aβ is first bound to the SA group of GM1 [76]. Several studies pointed out that His^{13} and Lys^{28} are important for the binding. Chemical shift changes were observed in the His^{13}–Leu^{17} region of Aβ-(1–40) upon binding to GM1 micelles in the presence of a physiological concentration of salt [77]. MD simulations revealed that the His^{13}–Gln^{15} segment of the protein recognized the artificial GM1-glycan cluster by non-specific stacking interaction between side chains of His and rings of sugar residues. The binding of Lys^{28} to SA triggered the helix formation at the C-terminus [78]. The His^{13}, Ser^{8}, and C-terminal hydrophobic residues (Leu^{34}, Val^{40}, and Ala^{42}) of Aβ-(1–42) formed H-bonds with the SA and GalNAc residues of GM1 in GM1/cholesterol/POPC (1/8/23) bilayers [79].

The hydrophobic interaction between the sugar CH groups and N-terminal part of Aβ may be important for binding. The binding affinity increases with the number of

sugar residues in sugar lipids, suggesting the involvement of Aβ–sugar interactions [67]. The results of MD simulations suggested the importance of a CH–π interaction between the aromatic side chains of Aβ and sugar carbohydrate moieties [75]. However, the substitution of Phe residues with non-aromatic cyclohexylananine (Cha) groups only marginally reduced binding, suggesting that aromaticity is not crucial for binding [80].

3.4. Structural changes of Aβ after binding to GM1 clusters

The structures of Aβ-(1–40) in the presence of lyso-GM1 and GM1 micelles were studied using NMR by the group of Kato. The His^{14}–Val^{21} and Ile^{31}–Val^{36} segments form helical structures in lyso-GM1 micelles (helicity 53%) [81] and the sugar–lipid interface is primarily perturbed [82]. A recent study, however, indicated that the former helix is destabilized in GM1 micelles and a monomeric hairpin structure is also formed by a helix-to-β-sheet transition [83]. At a Aβ-to-GM1 ratio of 0.067, the two C-terminal residues exhibit two distinct conformational states that are reactive with the amyloid-specific dye thioflavin T [84].

Both Aβ-(1–40) and Aβ-(1–42) eventually form amyloid fibrils on GM1 cluster-containing membranes via α-helix-rich structures and meta-stable β-sheet-rich oligomers (Fig. 3). The former conformations are adopted at low Aβ-to-GM1 ratios below ~0.01 [64, 66]. The helicity of membrane-bound Aβ-(1–40) was ~50% [85] in accordance with the NMR results [81]. An increase in the Aβ-to-GM1 ratio leads to the formation of the latter structure. A detailed analysis of ratio-dependent changes of CD spectra led to an estimation that the oligomer is composed of ~15 Aβ molecules. The pre-amyloid aggregate was stable for at least 8 days at 37°C [85]. The coexistence of α-helix and β-sheet structures was also observed by Raman spectroscopy [76].

A further increase in the Aβ-to-GM1 ratio above ~0.04 leads to the formation of amyloid fibrils (membrane fibrils) [85], and the structures we found for Aβ-(1–40) were completely different from those formed in aqueous solution (water fibrils) [86]. The structures of water fibrils have been extensively investigated mainly by solid state NMR (ssNMR), and they are essentially composed of in-register parallel β-sheets with the disordered N-terminal segment for both Aβ-(1–40) [87] and Aβ-(1–42) [88], although

detailed ternary structures depend on the method of preparation, such as with or without agitation. Fourier-transform infrared (FTIR) spectra supported this conclusion. Water fibrils exhibited an intense band typical of β-sheets at 1631.1 cm^{-1} without a higher wavenumber weak band, a hallmark of antiparallel β-sheets (Fig. 4A) [89]. In contrast, FTIR spectra of membrane fibrils featured antiparallel β-sheets with a minor band at around 1695 cm^{-1} in addition to a major β-sheet band at 1626.0 cm^{-1} (Fig. 4B). Transmission electron microscopy (TEM) revealed that membrane fibrils (~12 nm, Fig. 4D) were thicker than water fibrils (~8 nm, Fig. 4C). The height of water fibrils as measured by atomic force microscopy (AFM) was ~7 nm (Fig. 4C), in accordance with previous studies [87, 90]. In contrast, membrane fibrils had flat, tape-like structures composed of a single β-sheet layer, as judged by their extremely short height (~0.6 nm, Fig. 4D). We analyzed the structures of membrane fibrils by ^{13}C-edited FTIR spectra. The carbonyl group of Phe^{4}, Val^{12}, Phe^{19}, Phe^{20}, Val^{24}, or Val^{36} of Aβ-(1–40) was labeled with ^{13}C and fibrils were prepared using unlabeled or labeled Aβ. FTIR difference spectra (^{13}C – ^{12}C) clearly suggested that membrane fibrils possess β-sheet structures almost along the entire sequence, at least from Phe^{4} to Val^{36}. This

conclusion may explain the thickness of the fibrils (~12 nm) because the translation per residue value for β-sheets is ~0.33 nm [91].

Having confirmed that membrane fibrils were a single-layer β-sheet containing an antiparallel orientation, the next question was the inter-strand structure. We hypothesized that 2-residue-shifted antiparallel β-sheets were the most probable structures because they could be better stabilized by multiple salt bridges and hydrophobic/π–π interactions, the former being augmented in a less polar membrane environment. (Fig.5A). To confirm this model, chemical cross-linking experiments were carried out using G9C and A30C-Aβ-(1–40). The two Cys residues are expected to be in close proximity when the 2-residue-shifted antiparallel β-sheets are formed. Water fibrils produced mainly G9C- and A30C-Aβ homodimers as cross-linked products, in accordance with the in-resister parallel β-sheet structure model [87]. In contrast, in the case of membrane fibrils, not only the homodimers, but also significant amounts of the heterodimer, were detected, suggesting the coexistence of in-resister parallel and 2-residue-shifted antiparallel β-sheets. This conclusion was confirmed by non-perturbing ssNMR experiments using [^{15}N]F19, [1-^{13}C]A21-Aβ-(1-40), [^{15}N]L17,

[1-^{13}C]A21-Aβ-(1-40), and [^{15}N]V18, [1-^{13}C]A21-Aβ-(1-40) [86].

GM1 sugar groups containing both hydrophobic CH and hydrogen-bonding OH groups can accommodate this unique tape-like structure without sequestering hydrophobic residues. The in-resister parallel arrangement cannot be extended in the environment because electrostatic repulsion between adjacent charged residues with the same sign is enhanced in the low-polarity environment. Thus, a strand should be flipped, making a 2-residue-shifted antiparallel configuration, eventually forming an 'amyloid tape' structure composed of mixed in-register parallel and 2-residue-shifted antiparallel β-sheets (Fig. 5B). Indeed, similar amyloid fibrils are formed in a less polar 1, 4-dioxane/water mixture [92].

Matsubara et al. also investigated the interaction of 10 μM Aβ-(1–40) with supported planar bilayers composed of GM1/SM/cholesterol or galactosyl ceramide/SM/cholesterol by AFM and FTIR spectroscopy. Fibrils were more effectively generated in the former system. FTIR spectra of fibrils exhibited peaks at ~1635 and ~1695 cm^{-1}, which were assigned to parallel β-sheet and β-turn structures, respectively [93]. However, they could also be assigned to antiparallel β-sheet

structures [86].

4. Mechanisms of cytotoxicity

4.1. Monitoring self-assembly of Aβ on neuronal cells

The aggregational processes of Aβ on living cells and cytotoxic events were simultaneously monitored to elucidate the 'culprit' of cytotoxicity. Fluorescence correlation spectroscopy (FCS) can determine the size (diffusion coefficient) of a diffusing particle, which is an aggregate of fluorescently labeled Aβ in this case. The aggregation of Aβ-(1−42) doped with the HL647-labeled protein was monitored under fibril-forming conditions, i.e., 5 μM. The aggregate size increased to ~10 mers on average during 6–10.5-h incubation (Fig. 6A), after which the particles became immobile and were stained with Congo red, indicating the formation of amyloids. Similar results were observed for Aβ-(1−40), although a ten-times higher concentration was needed (Fig. 6A). Caspase-3, an effector caspase for apoptosis, was activated

only when amyloids were detected for both Aβ-(1−42) (Fig. 6B) [94] and Aβ-(1−40) (Fig. 6C) [95], suggesting that not oligomers but amyloids are responsible for cytotoxicity. In accordance with this, membrane fibrils formed in the presence of GM1 liposomes are also cytotoxic whereas water fibrils are not (Fig. 4E) [69, 92].

4.2. Signaling cascades involved in apoptosis

Although toxic soluble Aβ-(1–40) assemblies (200–300 kDa) are generated by incubation with limited concentrations of GM1 in raft-like liposomes [28], apoptosis mechanisms induced by membrane fibrils will be discussed here. Model membrane studies suggested that Aβ-(1–40) induced fibril-dependent fragmentation of membranes through a detergent-like mechanism in the presence of total ganglioside extract from the porcine brain [96]. However, cellular studies indicated that the mechanisms of cytotoxicity involve complex signaling cascades [95]. Inhibitor experiments suggested that the Toll-like receptor (TLR) 4/TLR 6–NFκB signaling pathway and Nod-like receptor family, pyrin domain-containing (NLPP) 3 inflammasomes were activated.

Upon amyloid formation, NFκB was translocated into the nucleus. An increase in the intracellular Ca^{2+} concentration as well as the generation of reactive oxygen species were also observed, suggesting mitochondrial damage. These did not occur when Aβ was in the oligomeric state. Knockout cell studies indicated that apoptosis was induced via a caspase 8 --> caspase 9 --> caspase 3 route. It should be noted that water fibrils did not evoke apoptosis at the same protein concentration.

5. Conclusion

Figure 7 summarizes Aβ-GM1 interactions. When GM1 molecules do not form clusters, Aβ remains in solution and does not bind to membranes ('healthy, state'). Once GM1 clusters are generated by endosomal dysfunction or changes in lipid metabolism (e.g., increase in cholesterol), Aβ interacts with them ('pathological state'), forming an α-helix rich structure (~50% helicity). With an increasing protein density, the helical form is converted to preamyloid β-sheet-rich oligomers comprising ~15 Aβ molecules. In contrast to oligomers formed in solution, these GM1-mediated

oligomers are nontoxic. A further increase in the Aβ density leads to the formation of unique, tape-like amyloid fibrils composed of a single layer of mixed in-register parallel and 2-residue-shifted antiparallel β-sheets. The membrane fibrils trigger apoptosis via complicated signaling cascades.

Since its discovery 25 years ago, extensive studies have clarified the involvement of GAβ in AD and revealed its underlying mechanisms. Yanagisawa's group recently developed a simple blood assay to estimate the amyloid burden in the brain [97]. Thus, it is possible to screen pre AD patients without cognitive decline and prevent their disease development. Multiple intervention strategies are possible, such as a decrease in the Aβ level with inhibitors for β- or γ-secretase, inhibition of GM1-mediated Aβ aggregation with small compounds [98], and prevention of apoptosis. Furthermore, the administration of GM1 may protect neurons from Aβ-induced adverse effects by sequestering Aβ [99-101]. It has yet to be explored how to modify the disease based on the GAβ cascade.

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Figure legends

Fig. 1 Aβs and gangliosides. (A) The amino acid sequences of APP around Aβ. The β- and γ-secretase cleavage sites are shown in red. Representative mutation sites in familial AD are shown in green. (B) Structures of major gangliosides in neurons.

Fig. 2 Differences in Aβ interaction between GM1-containing raft and non-raft membranes. (A) Binding of DAC-Aβ-(1–40) to GM1/SM/cholesterol (2:4:4, closed circles) and GM1/PC (2:8, open circles) bilayers at 37°C. Relative fluorescence enhancements of DAC-Aβ as defined by $(F - F_0)/F_0$, where F and F_0 denote fluorescence intensity in the presence and absence of liposomes, respectively, are plotted as a function of the lipid-to-DAC-Aβ ratio. Original numerical data were taken from Ref. {Kakio, 2002 #629}. (B) Amyloid formation by Aβ-(1–40), as detected with the amyloid specific dye thioflavin-T. Aβ-(1–40) (50 μM) was incubated with GM1/SM/cholesterol (2:4:4) and GM1/PC (2:8) bilayers (250 μM) at 37°C. for 19 h. Original numerical data were taken from Ref. {Okada, 2008 #917}. (C) Top views of

the snapshot structures of membranes composed of 48 GM1/96 SM/96 cholesterol (upper) and 48 GM1/192 PC molecules (lower) simulated by MD. The glycan and ceramide portions of GM1 are shown in red and yellow, respectively. SM/PC and cholesterol are drawn in green and blue, respectively. Reprinted with permission from J. Phys. Chem. B2012116175111-5121, Publication Date:April 12, 2012, https://doi.org/10.1021/jp207881k. Copyright 2012 American Chemical Society.

Fig. 3 Conformations of Aβs in the presence of GM1/SM/cholesterol (4:3:3) liposomes. Circular dichroism spectra of Aβs (15 μM) were measured at 37 ºC in the presence of the GM1-containg raft-like vesicles. Traces: blue, Aβ-(1–40) at a GM /Aβ ratio of 10; red, Aβ-(1–42) at a GM /Aβ ratio of 10, green, Aβ-(1–40) at a GM /Aβ ratio of 60; orange, Aβ-(1–42) at a GM /Aβ ratio of 60. Original numerical data were taken from Ref. {Ogawa, 2011 #948}.

Fig. 4 Characterization of water- and membrane-fibrils by Aβ-(1–40). FTIR spectra of (A) water- and (B) membrane-fibrils. TEM images (upper) and AFM height

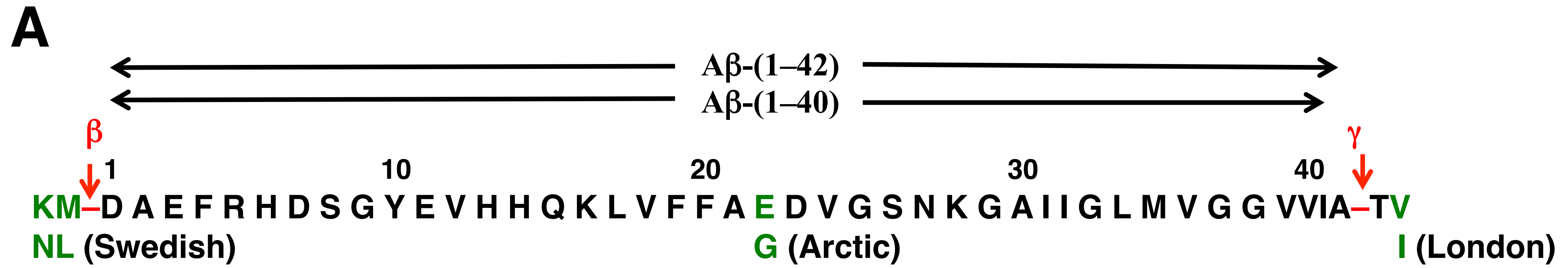
profiles (lower) of (C) water- and (D) membrane-fibrils. Scale bars show 50 nm. (E) Cytotoxicity of both types of fibrils against SH-SY5Y human neuroblastoma cells at 25 μM after a 24-h incubation at 37 °C (mean ± S.D., *n* = 6). * Two sample t-test. Reprinted with permission from *ACS Chem. Neurosci.*2019101563-572, Publication Date:October 11, 2018, https://doi.org/10.1021/acschemneuro.8b00424. Copyright 2018 American Chemical Society.

Fig. 5 Structure of membrane-fibrils by Aβ-(1–40). (A) A two-residue-shifted antiparallel β-sheet can be stabilized by multiple salt bridge and π–π interactions. G9 and A30 residues are expected to be located in close proximity. (B) A proposed model structure of membrane-fibrils. Reprinted with permission from *ACS Chem. Neurosci.*2019101563-572, Publication Date:October 11, 2018, https://doi.org/10.1021/acschemneuro.8b00424. Copyright 2018 American Chemical Society.

Fig. 6 Self-assembly of Aβ on and cytotoxicity against neuronal cells. (A) The

aggregation numbers of 5 μM Aβ-(1–42) (open circles) and 50 μM Aβ-(1–40) (closed circles) on SH-SY 5Y cells are plotted as a function of incubation time. Correlation between amyloid formation as detected by Congo red (red bars) and apoptosis as detected by the activation of caspase 3 (blue bars) for 5 μM Aβ-(1–42) (B) and 50 μM Aβ-(1–40) (C) as a function of incubation time. Mean ± S. E. ($n = 100$). * $p < 0.001$. Original numerical data were taken from (A, B) Ref. {Itoh, 2018 #1130} and (C) Takada et al, unpublished work.

Fig. 7 Summary of Aβ–GM1 interactions. Apoptosis-inducing 'toxic amyloid tapes' are eventually formed via an α-helix-rich form and nontoxic β-sheet-rich oligomer in the Aβ-to GM1 ratio on the membrane. See the text for details.



B

GM1

Gal GalNAc Gal Glc Cer SA

GD1a

Gal-GalNAc-Gal-Glc-Cer
SA SA

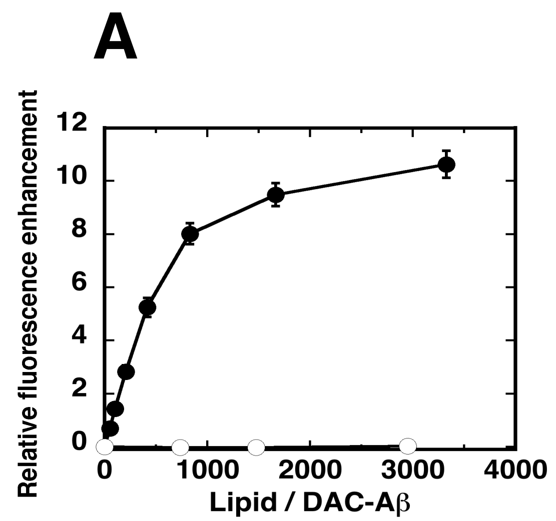
GD1b

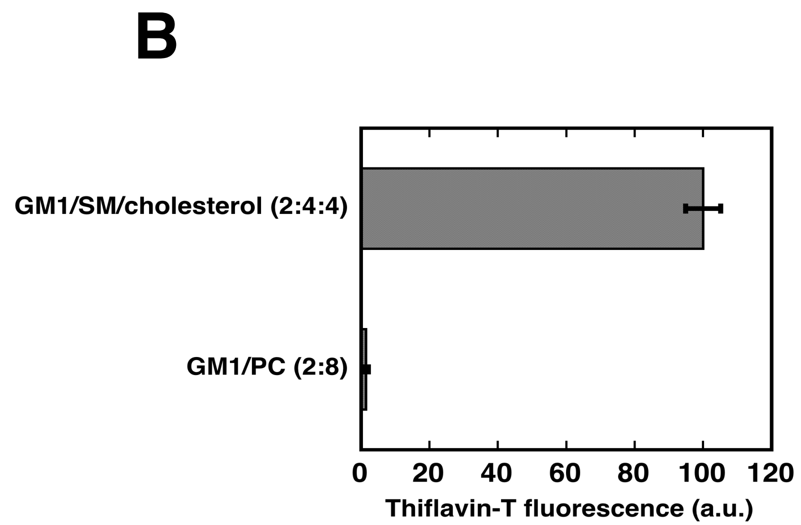
Gal-GalNAc-Gal-Glc-Cer
SA
SA

GT1b

Gal-GalNAc-Gal-Glc-Cer
SA SA
SA

Fig. 1 Matsuzaki





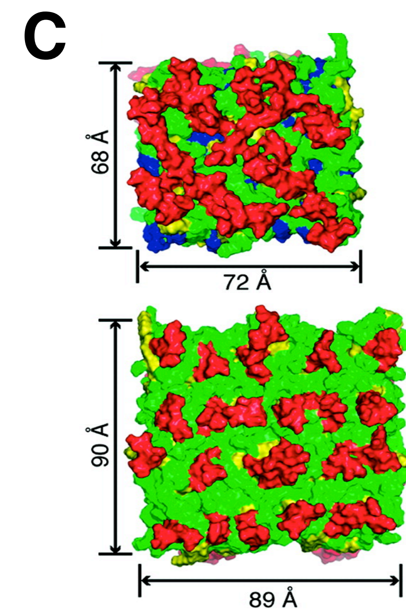


Fig. 2 Matsuzaki

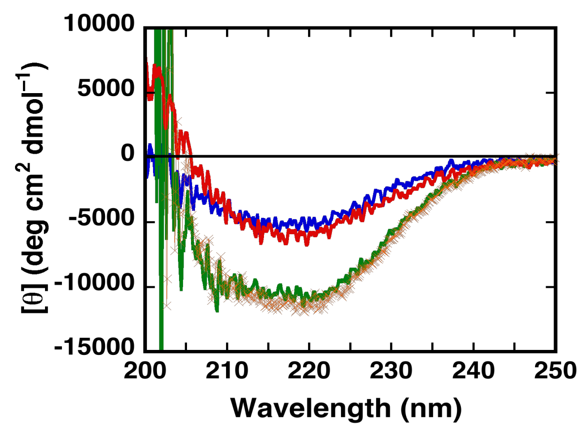


Fig. 3 Matsuzaki

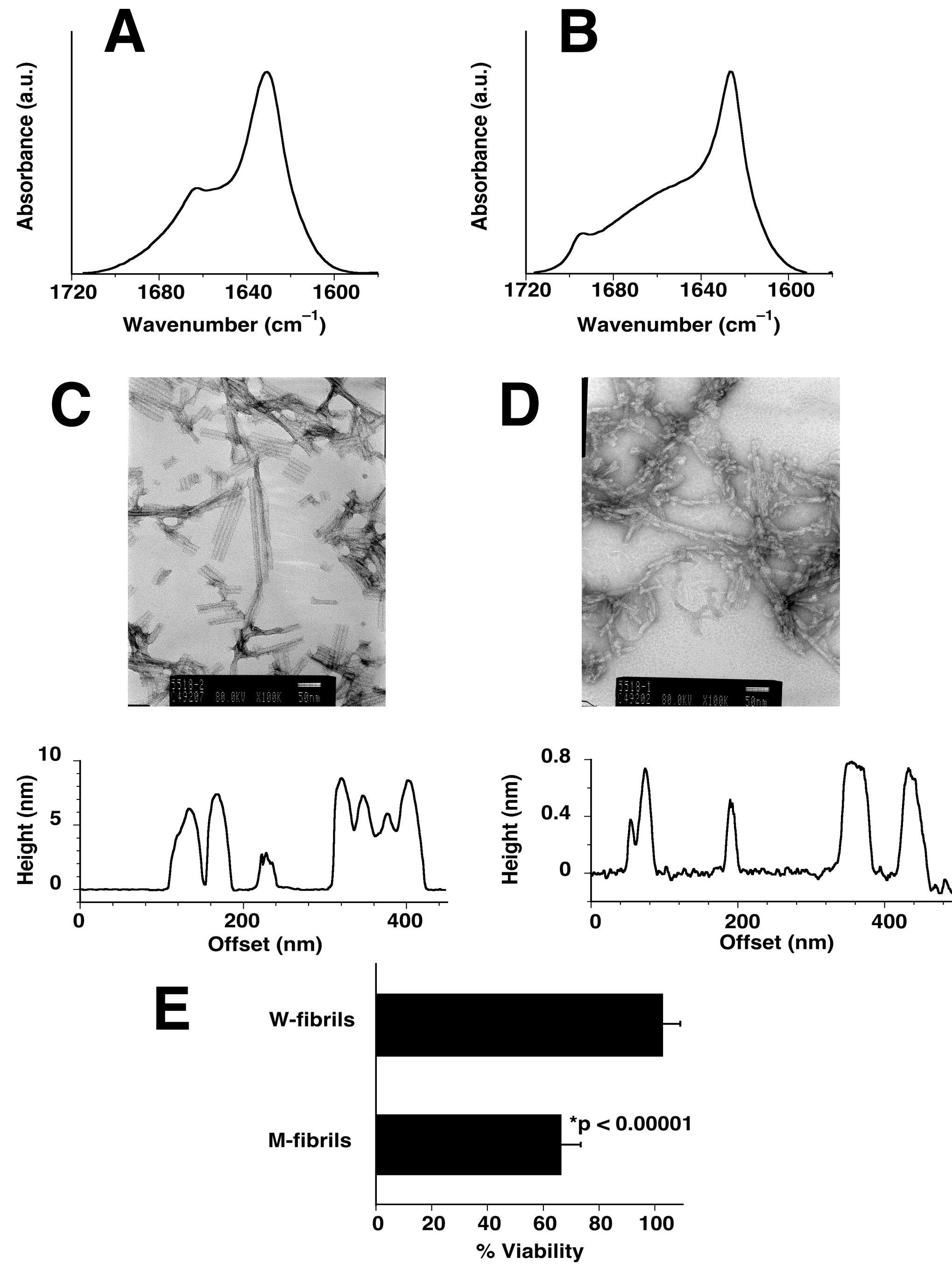
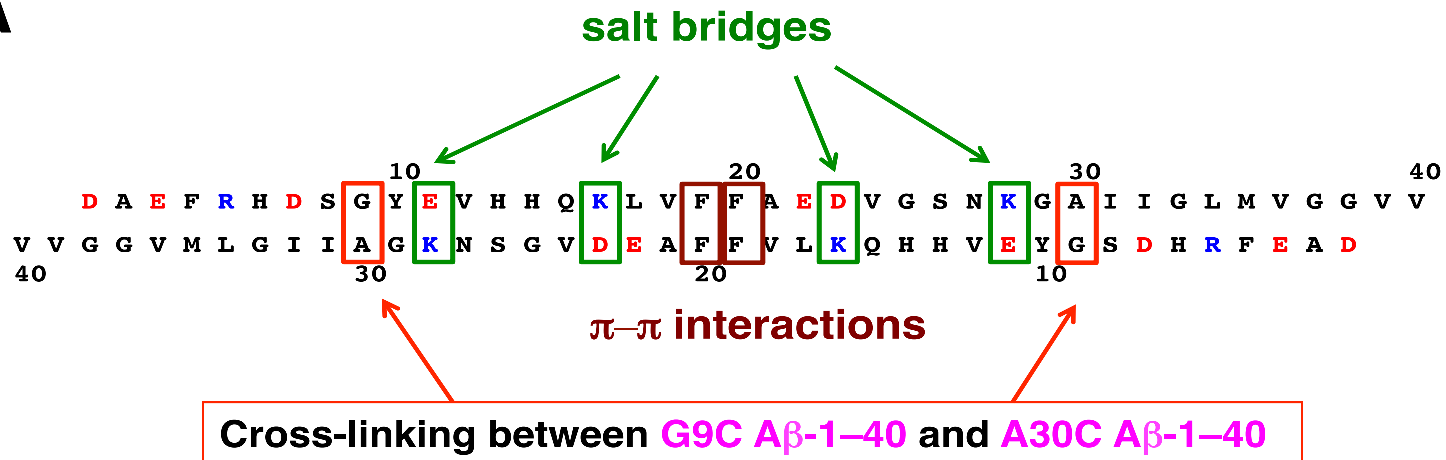


Fig. 4 Matsuzaki

A



B

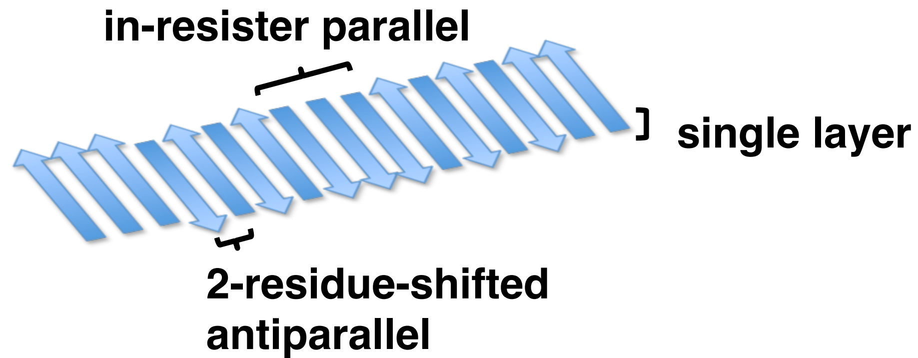
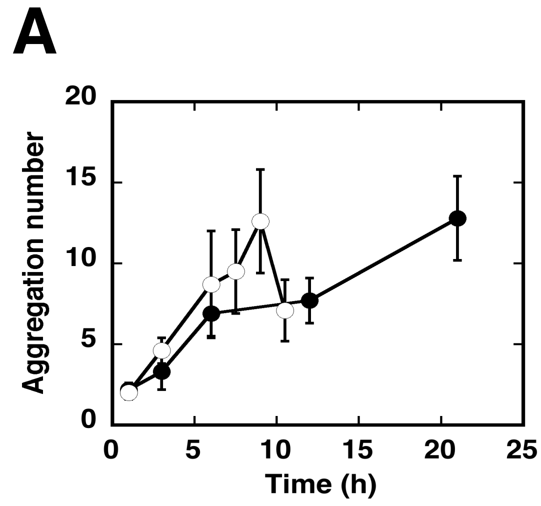
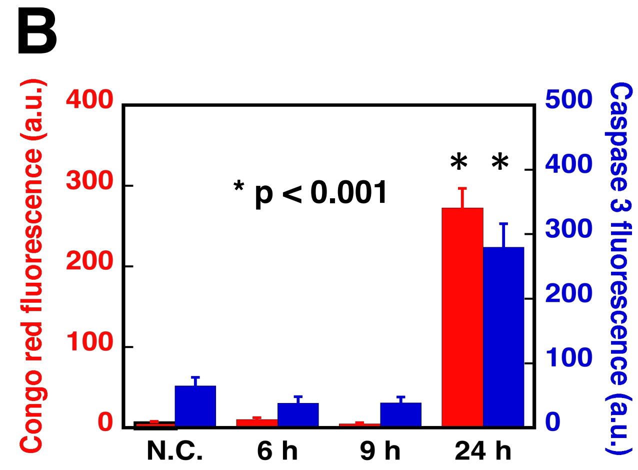


Fig. 5 Matsuzaki





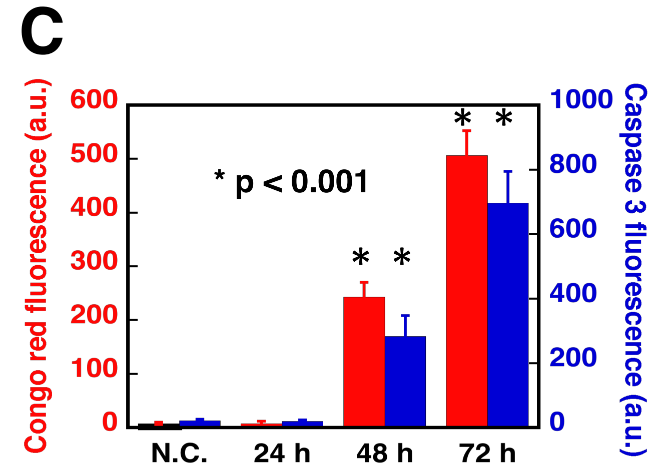


Fig. 6 Matsuzaki

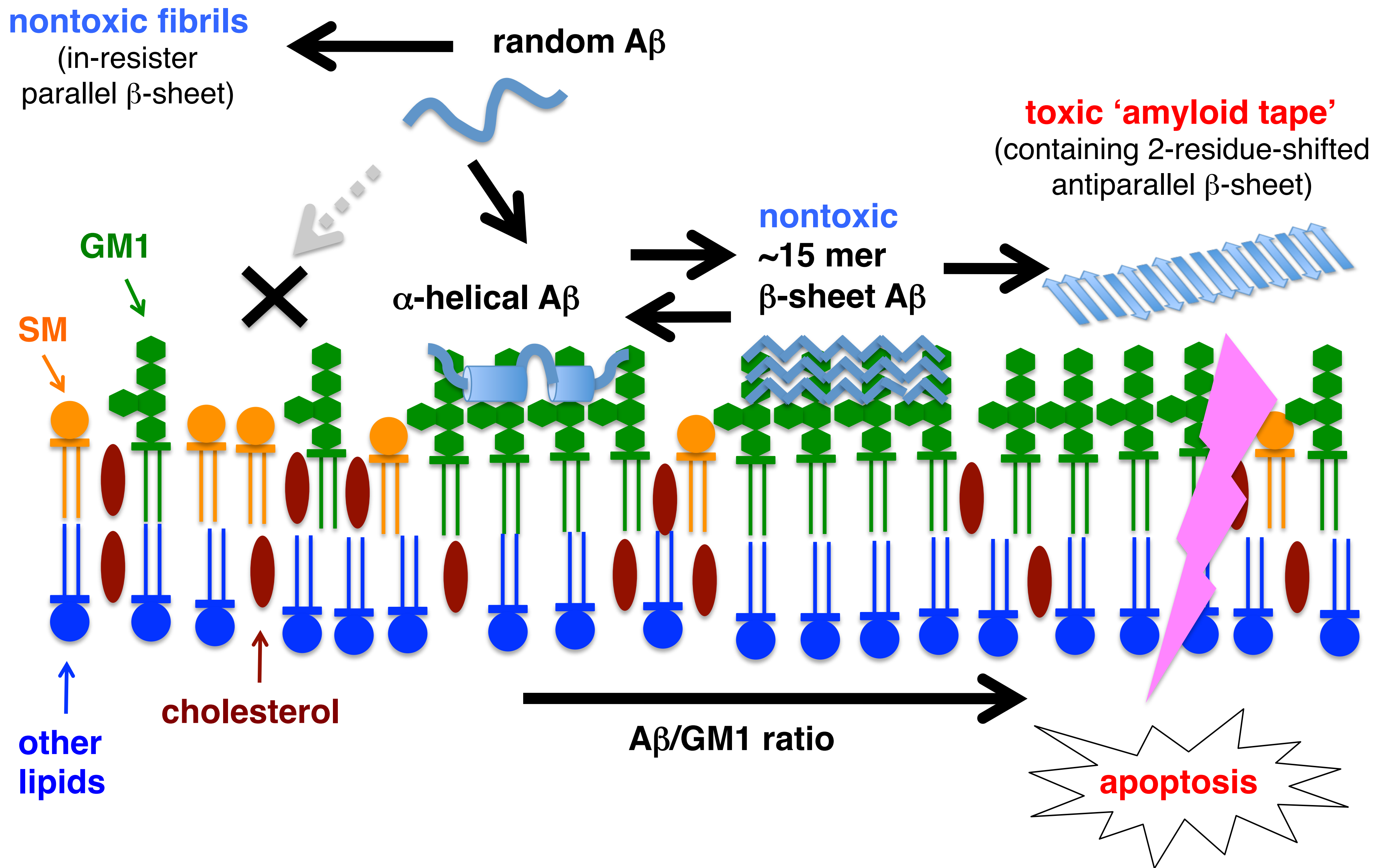


Fig. 7 Matsuzaki