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Amyloid β-peptide 1-42 modulates the proliferation of mouse neural stem cells: Up-regulation of fucosyltransferase-IX and Notch signaling

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Abstract

Amyloid β-peptides (Aβs) aggregate to form amyloid plaques, also known as senile plaques, which are a major pathological hallmark of Alzheimer's disease (AD). Aβs are reported to possess proliferation effects on neural stem cells (NSCs); however, this effect remains controversial. Thus, clarification of their physiological function is an important topic. We have systematically evaluated the effects of several putative bioactive Aβs (Aβ1-40, Aβ1-42, and Aβ25-35) on NSC proliferation. Treatment of NSCs with Aβ1-42 significantly increased the number of those cells (149±10 %). This was not observed with Aβ1-40 which did not have any effects on the proliferative property of NSC. Aβ25-35, on the other hand, exhibited inhibitory effects on cellular proliferation. Since cell surface glycoconjugates, such as glycolipids, glycoproteins, and proteoglycans, are known to be important for maintaining cell fate determination, including cellular proliferation, in NSCs and they undergo dramatic changes during differentiation, we examined the effect of Aβs on a number of key glycoconjugate metabolizing enzymes. Significantly, we found for the first time that Aβ1-42 altered the expression of several key glycosyltransferases and glycosidases, including fucosyltransferase-IX (FUT9), sialyltransferase-III (ST-III), glucosylceramide ceramidase (GLCC), and sialidase (Neu4). FUT9 is a key enzyme for the synthesis of the Lewis x carbohydrate epitope, which is known to be expressed in stem cells. Aβ1-42 also stimulated the Notch1 intracellular domain (NICD) by up-regulation of the expression of Musashi-1 and the paired box protein, Pax6. Thus, Aβ1-42 up-regulates NSC proliferation by modulating the expression of several glycogenes involved in Notch signaling.

Keywords

Alzheimer's disease; amyloid β-peptide; glycosyltransferase; glycogene; neural stem cell; cell proliferation

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Conflict of Interest
The authors declare no conflict of interest.

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Introduction

Alzheimer's disease (AD) is one of the most common forms of dementia with clinical symptoms that include deficits in memory, judgment, thinking, and behavior. These symptoms usually develop slowly and become worse over time, interfere with daily tasks, and ultimately lead to death. It is well accepted that deposition of aggregated amyloid β-protein (Aβ) to form amyloid plaques, also known as senile plaques, together with associated reactive astrocytosis and dystrophic neuritis, represent major pathological hallmarks of AD [3]. There are two dominant forms of Aβs, Aβ1-40 and Aβ1-42. Both have a high tendency to assemble initially into the soluble form, and later to form insoluble aggregated fibrils as extracellular amyloid plaques in the AD brain. Intermediate soluble oligomers of Aβs, rather than the aggregated Aβs, are increasingly recognized as having cellular toxicity in Alzheimer's disease [4,5].

Neural stem cells (NSCs) are undifferentiated neural cells characterized by their high proliferative potential and the capacity for self-renewal with retention of multipotency, i.e., generating brain-forming cells such as neurons, astrocytes, and oligodendrocytes. Our previous *in vitro* study showed that soluble Aβ1-42 dramatically increased the number of NSCs, but soluble Aβ1-40 did not [6]. Both the aggregated forms of Aβ1-40 and Aβ1-42 promoted the proliferation of NSCs, but soluble and aggregated Aβ25-35 showed inhibitory effects. As well as pathological conditions, it has been also reported that Aβs are produced by cultured cells during normal cellular metabolism [7,8]. Because Aβ1-40 and Aβ1-42 are present in the brain and cerebrospinal fluid of normal individuals, it suggests that these peptides are likely physiologically active in normal life [9]. Despite extensive efforts for studying Aβs for their cytotoxic effects in AD, the normal biological functions and positive effects of Aβs have remained elusive.

Gangliosides are sialic acid-containing glycosphingolipids (GSLs) expressed primarily in the outer leaflet of the plasma membrane of all vertebrate cells and are particularly abundant in the nervous system [10,11]. The expression of neural gangliosides changes dramatically during cellular differentiation and brain development. For instance, in rodent brains a shift from the synthesis of simple gangliosides, such as GM3 and GD3, to the synthesis of the more complex gangliosides in the a- and b-series during brain development has been well documented [12,13]. A2B5 monoclonal antibody was reported [14] in 1979, and it recognizes c-series gangliosides including GQ1c, GT1c and GT3 [15,16] and to some extent sulfatide [17]. The c-series ganglioides are abundant in embryonic mammalian brains, but not in adult brain [18,19]. During development, A2B5 antigens are expressed in glial precursor cells [20]. Significant changes in ganglioside patterns in AD brains have been reported; major gangliosides such as GM1, GD1a, GD1b and GT1b are all decreased in AD patients brain and b-series gangliosides such as GD1b and GT1b are preferentially affected [21–25]. Additionally, in frontal and parietal cortex simple gangliosides, including GM2, GM3, GM4 and GD3, are elevated. These findings suggest that abnormal ganglioside metabolism coincides with the affected brain region of neurodegeneration in AD patients. In AD mouse brain, it was reported that GT1a, GD1a and GQ1b are slightly decreased and there are increases of minor gangliosides such as GM2, GM3 and GD3 in the cerebral cortex [26]. Most interestingly, we have reported there is an increase of cholinergic neuronal

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marker gangliosides such as GT1aα and GQ1bα in AD mouse brain [27]. *In vitro* experiments also revealed that hippocampal neuronal cultures treated with Aβ25–35 showed enhanced metabolism of lipids such as gangliosides and phospholipids [28]. In addition, exposure of rat cultured cortical neurons to Aβ25–35 induced a substantial increase of the intracellular GD3 levels [29]. Those reports suggest that Aβs can modulate ganglioside metabolism in neural cells and prompted us to hypothesize that Aβs could alter glycogene expression in NSCs to account for the ganglioside changes.

Stage-specific embryonic antigen-1 (SSEA-1/Lewis X/CD15) is a well-known carbohydrate antigenic epitope of undifferentiated cells and has been recognized as an NSC marker [30]. The 3-fucosyl-*N*-acetyllactosamine or Lewis X carbohydrate structure is defined as [Galβ1-4(Fucα1-3)GlcNAcβ-]. Lewis X carbohydrate epitope is synthesized by transferring a fucose residue from GDP-fucose to *N*-acetylglucosamine (GlcNAc) by the action of α1,3-fucosyltransferase-IX (FUT9) [31]. Recently, we showed that FUT9 knockdown in mouse NSCs impaired Musashi-1 expression and NSC proliferation [32], suggesting that NSC proliferation can be modulated by FUT9 and the Notch signaling pathway.

In this study, we investigated the possibility that proliferation of NSCs stimulated by Aβ1-42 is associated with altered glycogene expression in the NSCs. Here we demonstrated that Aβ1-42 significantly activated the expression of several enzymes, including fucosyltransferase-IX (FUT9), sialyltransferase-III (ST-III), glucosylceramide ceramidase (GLCC), and sialidase (Neu4). Aβ1-42 also markedly activated several NSC markers, such as paired box protein (Pax6), Musashi-1 and Notch1 intracellular domain (NICD). Our present study thus provides a novel mechanism for the up-regulation of Musashi-1 expression with an increase of FUT9 to account for the proliferative effect of Aβ1-42.

Materials and Methods

Materials

Aβ1-40, Aβ1-42 and Aβ25-35 were purchased from Bachem Americas (Torrance, CA). Freshly prepared soluble Aβs were used for the experiments. Antibodies used were as follows: anti-Notch-1 (mouse) (BD Biosciences, San Jose, CA), and anti-actin (rabbit) (Sigma-Aldrich, St. Louis, MO)

Neural stem cell culture

Mouse NSCs were prepared from embryonic brains in the form of neurospheres, which were floating clonal aggregates formed by NSCs *in vitro* [33]. In brief, single-cell suspensions prepared from the striata of embryonic mouse brains [embryonic day (E)-14.5] by mechanical trituration were cultured in Neurobasal A medium (Life Technologies, Carlsbad, CA) supplemented with B27 (Life Technologies) and 20 ng/ml of basic fibroblast growth factor (Peprotech, Rocky Hill, NJ) and 20 ng/ml of epidermal growth factor (Peprotech). Neurospheres formed after 1 week were collected for further passages and analyses. The use of animals for this study was approved by the Institutional Animal Care and Use Committees at Georgia Regents University and the VA Medical Center, Augusta, GA.

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WST-8 assay

The number of NSCs cultured in the presence or absence of Aβs was estimated by the WST-8 assay using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The dissociated NSCs from neurospheres were plated at a density of 1 x 10^4 cells per well onto 96-well plates that had been coated with poly-L-ornithine (Sigma-Aldrich) and fibronectin (Sigma-Aldrich). On the next day, Aβs dissolved in media were added to each well. The total volume of the culture media was 100 μl/well. After 3 days of culture, 10 μl of WST-8 solution was added to each well. After incubating for 3 hours in a CO_2 incubator, the spectrophotometric absorbance of WST-8-formazan produced by the dehydrogenase activity in the living neural cells was measured at the wavelength of 450 nm using a Benchmark Plus Microplate Spectrophotometer (Bio-Rad Laboratories, Hercules, CA). The spectrophotometric absorbance measured by this assay was highly correlated with the number of living NSCs.

Reverse transcription-polymerase chain reaction

Total RNA samples were isolated from cultured NSCs using the Trizol reagent (Life Technologies). cDNAs were synthesized based on the total RNAs as templates using MultiScribe™ Reverse Transcriptase (Applied Bioscience). PCR was performed using ReadyMix™ REDTaq® (Sigma-Aldrich) with the following settings: 94°C for 5 min; 26–32 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. The primer sets used for PCR analysis are shown in Table 1. PCR products were analyzed by agarose gel electrophoresis using 1.5% agarose gels containing SYBR Safe™ DNA Gel stain (Life Technologies). The bands were quantified using the NIH ImageJ 1.46r image processing program (rsb.info.nih.gov) to reflect the original mRNA levels. Densitometric data were normalized against G3PDH (glycerol-3-phosphate dehydrogenase), a ubiquitously expressed enzyme catalyzing an essential step of glycolysis, mRNA. The normalized value from control (vehicle) is defined as 1.0.

Western blotting

NSCs were washed with PBS, lysed in RIPA buffer containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM NaF, 1 mM Na_3VO_4, 1% NP-40, 0.5% sodium deoxycholate, and 1% SDS, pH 7.5, supplemented with a complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN), and then centrifuged at 12,000 × *g* and 4°C for 10 min. Supernatants (cell lysates) were collected and the protein concentrations were measured using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific Inc., Rockford IL). Proteins were separated by SDS-PAGE (8 % gel) under reducing conditions and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were probed with primary antibodies followed by appropriate secondary antibodies conjugated with horseradish peroxidase (HRP, BD Biosciences). Signals were visualized with Western Lightning Western blot chemiluminescence reagent (Perkin Elmer Life and Analytical Sciences, Boston, MA). The bands were quantified using the NIH ImageJ. Densitometric values were normalized by setting the NICD/Actin protein ratio for control (vehicle) in each treatment. The normalized value from control (vehicle) is defined as 1.0.

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Statistical evaluation

Data are expressed as means ± standard deviation (SD) from three to eight independent experiments. Statistical significance was determined using unpaired two-tailed *t*-test and $p<0.05$ was regarded as significant.

Results

Increased number of NSCs by treatment with Aβ1-42

To assess the effect of the two major Aβ peptides (Fig. 1A) on NSCs, isolated NSCs were plated, Aβ1-40 or Aβ1-42 was added the following day, and living cells were measured 3 days after treatments. We measured the number of NSCs by the WST-8 assay; the spectrophotometric absorbance measured by this assay is known to be highly correlated with the number of living NSCs [34,35]. The relative absorbance of the WST-8 assay revealed the percentage of absorbance vs. vehicle treatment. We confirmed that treatment of NSCs with 10 μM of Aβ1-42 significantly increased the number of cells (149 %). This proliferative effect was observed only with Aβ1-42 but not with Aβ1-40 (10 μM). Aβ25-35, on the other hand, exhibited inhibitory effects on cellular proliferation (data not shown). These results are consistent with our previous study [6] and that by others [36–39] that showed Aβ1-42 promoted NSC proliferation. In the previous study [6], TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) assay, an indicator of cell death accompanied by DNA fragmentation, revealed that there were no significant TUNEL-positive cells in the intact cells and NSCs treated with the soluble forms of Aβs. This result was also consistent with our previous report that showed the expression of active caspase 3 was not different in intact cells and NSCs treated with the soluble form of Aβ1-40 [40]. These treatments of Aβ1-40 and Aβ1-42 were not toxic for NSCs in this culture system. Taken together, it suggests that the increase of cell number with soluble Aβ1-42 was likely caused by cell proliferation rather than by protection the cells from undergoing apoptosis.

Alteration of glycosyltransferase and glycosidase gene expressions by Aβs

Cell surface glycoconjugates are known to be important for maintaining stem cells, and their expression is dramatically altered during differentiation [10,11]. The expression of gangliosides changes remarkably during neuronal differentiation and brain development [12,13]. The expression of gangliosides is regulated primarily by glycosyltransferases and glycosidases (Fig. 2). For this reason, we analyzed the gene expression patterns of several key glycosyltransferases and glycosidases in NSCs treated with Aβ1-40 or Aβ1-42. Most of the glycosyltransferases involved in ganglioside synthesis, such as ceramide glucosyltransferase (GlcT), galactsyltransferase (GalT-1), sialyltransferase-I (ST-I), ST-II (GD3-synthase), N-aetylgalactosaminyltransferase (GalNAcT, GM2/GD2-synthase), and GalT-II, showed no significant difference after treatment by the two Aβs. On the other hand, the expression of ST-IV, ST-V and β-N-acetylhexaminidase β-subunit (HEXβ) increased by treatment with the two Aβs (Fig.3). We found that treatment with Aβ1-42 greatly increased the expression of certain glycosyltransferases, specifically, FUT9, sialyltransferase-III (ST-III), and glycosidases including glucocerebrosidase (GLCC), mitochondrial sialidase (Neu4) (Fig. 3). Treatment with either Aβ1-40 or 1-42 decreased the expression of ST-VII, β-N-acetylhexaminidase α-subunit (HEXα), and GM2 activator protein (GM2A). Our results

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thus show that Aβ treatment had a profound effect on the expression pattern of specific glycogenes in NSCs. FUT9 is not involved in ganglioside metabolism, but is a key enzyme for the synthesis of Lewis X-carrying N-glycans, which has been utilized as an NSC marker. In our previous study, FUT9 was shown to be expressed in NSCs and down-regulated in differentiated cells [41]. Increasing the expression of this key enzyme, FUT9, by Aβ1-42 is expected to affect NSC proliferation.

Expression of specific NSC markers

Next, we performed RT-PCR experiments and evaluated if Aβs affected the differentiation state of NSCs. Fig. 4A shows that Aβ treatments did not change the expression of the mRNA of a neuronal marker (MAP2), glial marker (GFAP), and an NSC marker, nestin, in NSCs. These data suggest that Aβ treatment did not affect the differentiation stages of NSCs. However, we found that the protein expression of the Notch1 intracellular domain (NICD) was up-regulated after Aβ1-42 treatment (Fig. 4B). Since we found FUT9 was also up-regulated after Aβ1-42 treatment, we postulated that the regulator of FUT9 should also be affected. Pax6 is known to promote proliferation of neural progenitor cells and neurogenesis [42]. Since it was reported that Pax6 controls the expression of FUT9 in the embryonic brain of rodents [43], we next analyzed PAX6 mRNA expression. This is consistent with the fact that Aβ1-42 increased Pax6 expression as revealed by our RT-PCR experiment. Recently, we reported that FUT9 regulates the proliferation of NSCs via modulation of the expression of Musashi-1 [32]. Musashi-1 plays a crucial role in maintaining the undifferentiated state of NSCs via activation of the Notch signaling pathway [44,45]. Numb protein was known to bind Notch-1 protein and inhibit the activation of the Notch signaling pathway. On the other hand, Musashi-1 protein binds to Numb mRNA and inhibits its translation, and Musashi-1 enhances Notch signaling by inhibiting Numb translation [45]. Therefore we performed an RT-PCR experiment and examined Musashi-1 expression after Aβ treatment. Intriguingly, Aβ1-42 increased Musashi-1 gene expression in NSCs (Fig. 4B). Our data thus are consistent with the following sequence of events: Aβ1-42 stimulated Notch signaling, which is followed by increased expression of Pax6, FUT9 and Musashi-1, and regulation of NSC proliferation.

Discussion

The present study provides the first evidence that soluble Aβ1-42 promotes NSC proliferation by modulation of FUT9 and Notch signaling. We and others have previously reported that soluble Aβ1-42 stimulated NSC proliferation. Soluble Aβ1-40, on the other hand, has no effect on NSC proliferation. The mechanism of NSC proliferation stimulated by Aβ1-42, however, is not well understood. For this reason, we investigated the potential mechanisms of Aβ1-42-stimulated NSC proliferation. Two major forms of Aβs, Aβ1-40 and Aβ1-42, are known (Fig. 1A). During normal metabolism of amyloid protein (APP), Aβ1-40 is abundantly produced [46]; however, Aβ1-42 is the major peptidic constituent of amyloid plaques. *In vitro*, freshly dissolved Aβ1-42 generates more stable dimer formation than does Aβ1-40 [47]. Aβ1-42 has less configurational entropy and is found to confer higher rigidity on the C-terminus, which may explain its higher amyloidogenic ability [48]. Yang and Teplow [49] reported that the addition of two terminal hydrophobic residues, isoleucine and

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alanine, on Aβ1-42, significantly increases the contact between the C-terminus and the central hydrophobic clusters. This additional hydrophobic interaction significantly stabilizes the β-sheet structure of Aβ1-42 as compared with that for Aβ1-40. These structural characteristics of Aβ1-42 may affect its interaction with the cell membrane and consequently its activity for promotion of NSC proliferation, as revealed by our observation.

With respect to Aβ 25–35, this peptide is considered as having the functional and responsible domain for the neurotoxic properties of Aβs [50]. Both Aβ1–42 and Aβ25–35 are known to be incorporated into phospholipid membranes. In the model lipid membrane system, the incorporated Aβ1–42 tends to destabilize the lipid bilayer, whereas Aβ25–35 inserted into the hydrophobic acyl-chain region of the phospholipid membrane to enhance membrane stability [51,52]. Treatment of Aβ 25-35 (50 μM) induces rapid cell lysis of red blood cells, whereas exposure of red blood cells to the same concentration of Aβ1–42 induces no significant lysis [53]. It has also been reported that Aβ 25-35 is more neurotoxic and causes more membrane protein oxidation than does Aβ1-42 [54]. The oxidation of the methionine residue in the peptide is believed to proceed through a free radical intermediate [55]. Substitution of methionine 35 in Aβ25-35 inhibits the aggregation, apoptotic effects, and neurotoxicity of the peptide [56,57]. Intriguingly, Aβ25–36 which has one additional amino residue on Aβ25-35 is no longer toxic to cultured neurons [54]. These observations on the property of Aβ25-35 are consistent with our finding that treatment of NSCs with Aβ25-35 decreased the number of NSCs.

Cell surface glycoconjugates, including glycoproteins, glycolipids, and proteoglycans, are known to play important functional roles in NSC maintenance. These carbohydrate-containing antigens serve as excellent cell surface biomarkers at various stages of cellular differentiation. A2B5 antibody recognizes c-series gangliosides, including GT3, GT1c and GQ1c [15]. These c-series gangliosides are abundant in fish brains and embryonic mammalian brains [58,59]. In embryonic brain and spinal cord, A2B5 antigens are expressed in glial precursor cells [20]. We found that the expression of ST-III, which is a key enzyme for the synthesis of c-series gangliosides, increased after Aβ1-42 treatment. This result suggests that the up-regulated ST-III should promote the increased synthesis of c-series gangliosides, which are expressed in embryonic brain. It is intriguing to suggest that Aβ1-42 treatment might promote proliferation as evidenced by the increased expression of neural progenitor cell (NPC) markers as observed in the current study.

Mouse Neu4 sialidase is predominantly expressed in brain [60]. In Neuro2A cells, the level of Neu4 gene expression decreases during retinoic acid-induced neuronal differentiation [61]. Trinngali *et al.* reported that overexpression of the Neu4 gene enhances an undifferentiated stem cell-like phenotype and cell proliferation in human neuroblastoma cells [62]. We have also confirmed that proliferating mouse NSCs, NPCs, and glial progenitor cells highly express Neu4 mRNA (data not shown) in culture. On the other hand, Neu4 expression decreases during neuronal and glial differentiation (data not shown). Thus, our data that Aβ1-42 stimulated Neu4 expression is consistent with those observations and may contribute to NSC proliferation and inhibit differentiation.

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We also found changes in gene expression in NSCs treated with Aβ1-40 unaccompanied by a proliferative effect. These altered glycogenes are not considered to regulate cell proliferation *per se*. In AD patient and AD mouse brains, it has been reported that there are significant changes in the ganglioside expression pattern. GM1, GD1a, GD1b and GT1b are all decreased in AD patient brain, and GT1a, GD1a and GQ1b are slightly decreased in AD mouse brain [26,21–25]. Additionally, simple gangliosides such as GM2, GM3 and GD3 are elevated. Altered glycogene expression can be considered to contribute to the observed changes in ganglioside composition. For example, decreased complex gangliosides and increased simple gangliosides in AD brain might be related to the up-regulation of HEXβ and desialylations from complex gangliosides. Increased expressions of ST-IV and ST-V should contribute to increased levels of complex gangliosides, such as GT1a, GD1a and GQ1b that are decreased in AD brain. However, evaluation of the precise functions of the altered glycogenes *in vivo* would require further studies.

A monoclonal antibody (mAb) against SSEA-1/CD15 was first established by immunization with F9 embryonic carcinoma cells [63]; subsequently the antigenic oligosaccharide structure that contained the Lewis X structure was established as Galβ1-4(Fucα1-3)GlcNAcβ-[64]. In a previous study [41], we found that the mRNA expression level of FUT9 was down-regulated in cells that were differentiated from NSCs. Recently we reported that Lewis X-carrying N-glycans, which account for 20% of the total N-glycans in NSCs, disappeared during differentiation [32]. By knocking down a FUT9 with a short interfering RNA (RNAi), we reported that the Lewis X-carrying *N*-glycans were actively involved in proliferating NSCs via modulation of the expression level of Musashi-1[32]. In the current study, we found that Aβ1-42 up-regulated the expression of Pax6, which is known to control FUT9 expression. The increased Pax6 and FUT9 stimulated by Aβ1-42 resulted in up-regulation of Musashi-1 expression. Furthermore, Aβ1-42 enhanced the amount of NICD with increased NSC proliferation. Taken together, our findings provide insights to the functional role of Aβ1-42 as an active modulator of glycogenes and Notch signaling for promotion of NSC self-renewal and proliferation.

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Abbreviations

AD Alzheimer's disease

Aβ amyloid β-protein

FUT9 fucosyltransferase-IX

NSC neural stem cell

TUNEL terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling. Gangliosides are abbreviated using the nomenclatural rules of IUPAC-IUB [1] and according to Svennerholm [2].

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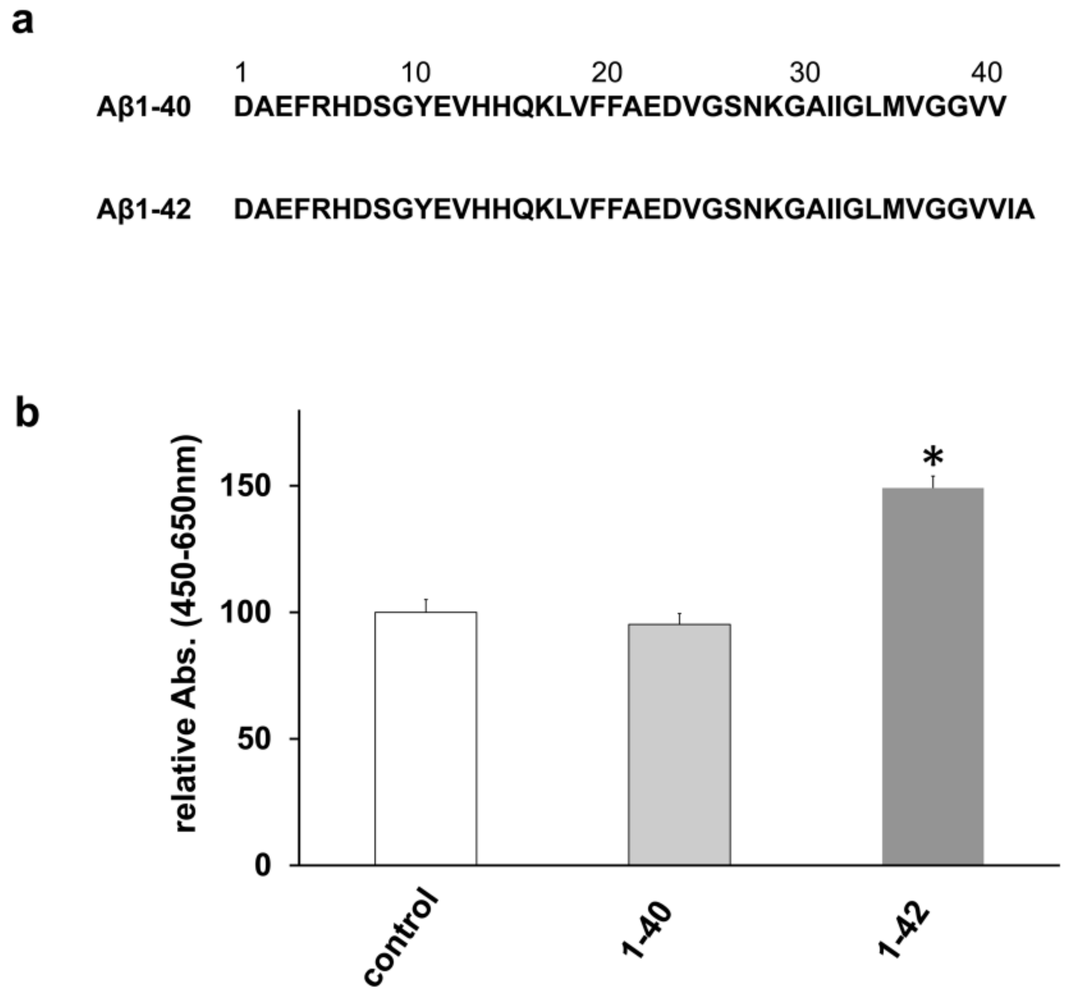

Fig. 1. Effects of soluble Aβs (Aβ1-40 or Aβ1-42) on mouse NSCs in culture

a. Primary structures of Aβ peptides. **b.** NSCs were cultured for 3 days with or without Aβs. The number of NSCs in the monolayer culture in the presence of soluble Aβs (10 μM) was estimated by the WST-8 assay. The y-axis represents relative absorbance (Abs.), which represents the percentage of absorbance against vehicle treatment. Each bar represents mean ± SD of 8 independent experiments (n = 8). Comparison was made between vehicle versus each treatment. *p<0.05.

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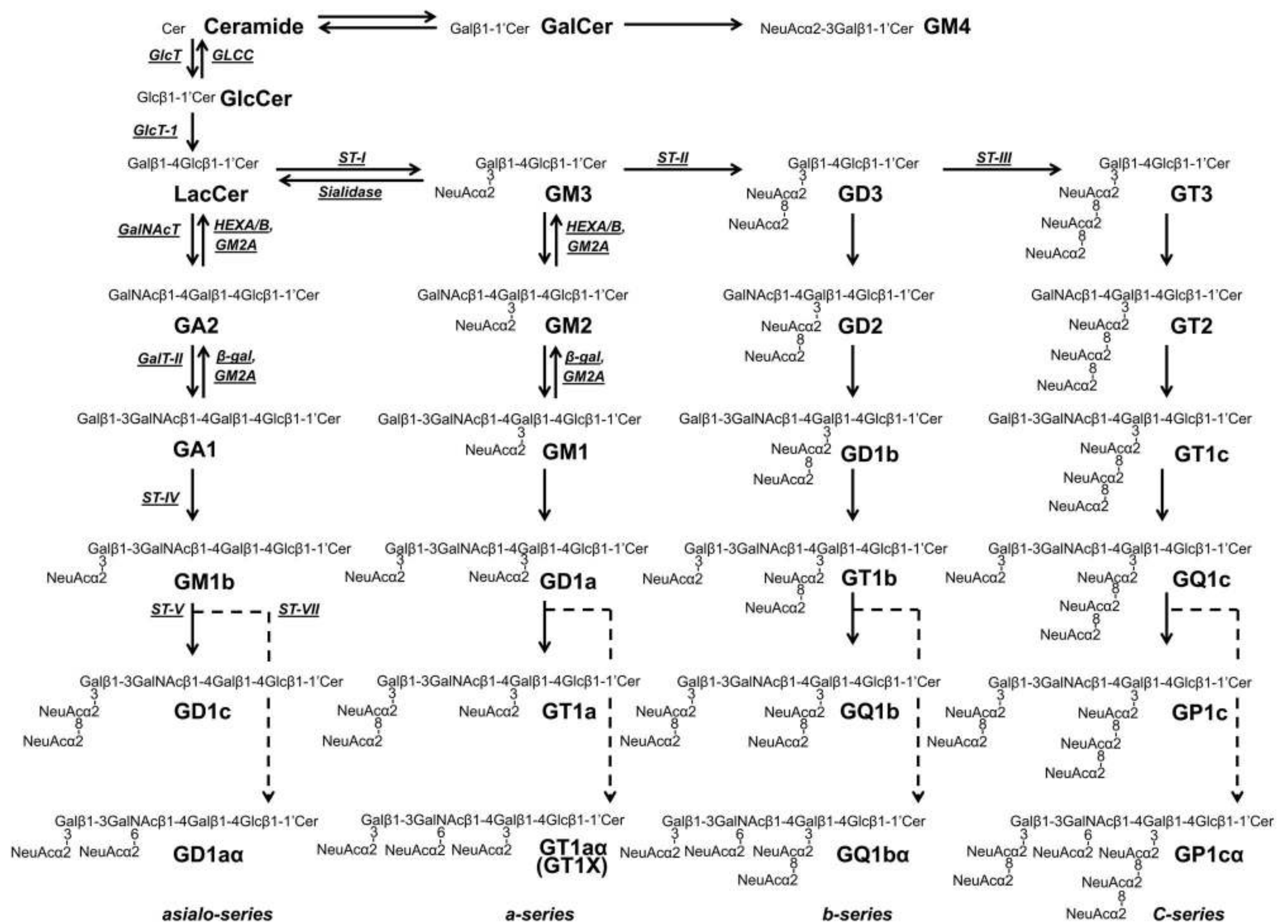

Fig. 2. Structures and metabolic pathways of gangliosides

The nomenclature for gangliosides and the components are based on those of Svennerholm (1981) and IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (1997). β-gal, lysosomal acid β-galactosidase; GalNAc-T, N-acetylgalactosaminyltransferase I (GA2/GM2/GD2/GT2-synthase); GalT-I, galactosyltransferase I (lactosylceramide synthase); GalT-II, galactosyltransferase II (GA1/GM1/GD1b/GT1c-synthase); GLCC, glucosylceramidase; GlcT, glucosyl transferase (glucosylceramide synthase); GM2A, GM2 activator protein; HEX, β-N-acetylhexosaminidase; ST-I, sialyltransferase I (GM3-synthase); ST-II, sialyltransferase II (GD3-synthase); ST-III, sialyltransferase III (GT3-synthase); ST-IV; sialyltransferase IV (GM1b/GD1a/GT1b/GQ1c-synthase); ST-V, sialyltransferase V (GD1c/GT1a/GQ1b/GP1c-synthase); ST-VII, sialyltransferase VII (GD1aα/GT1aα/ GQ1bα/GP1cα-synthase).

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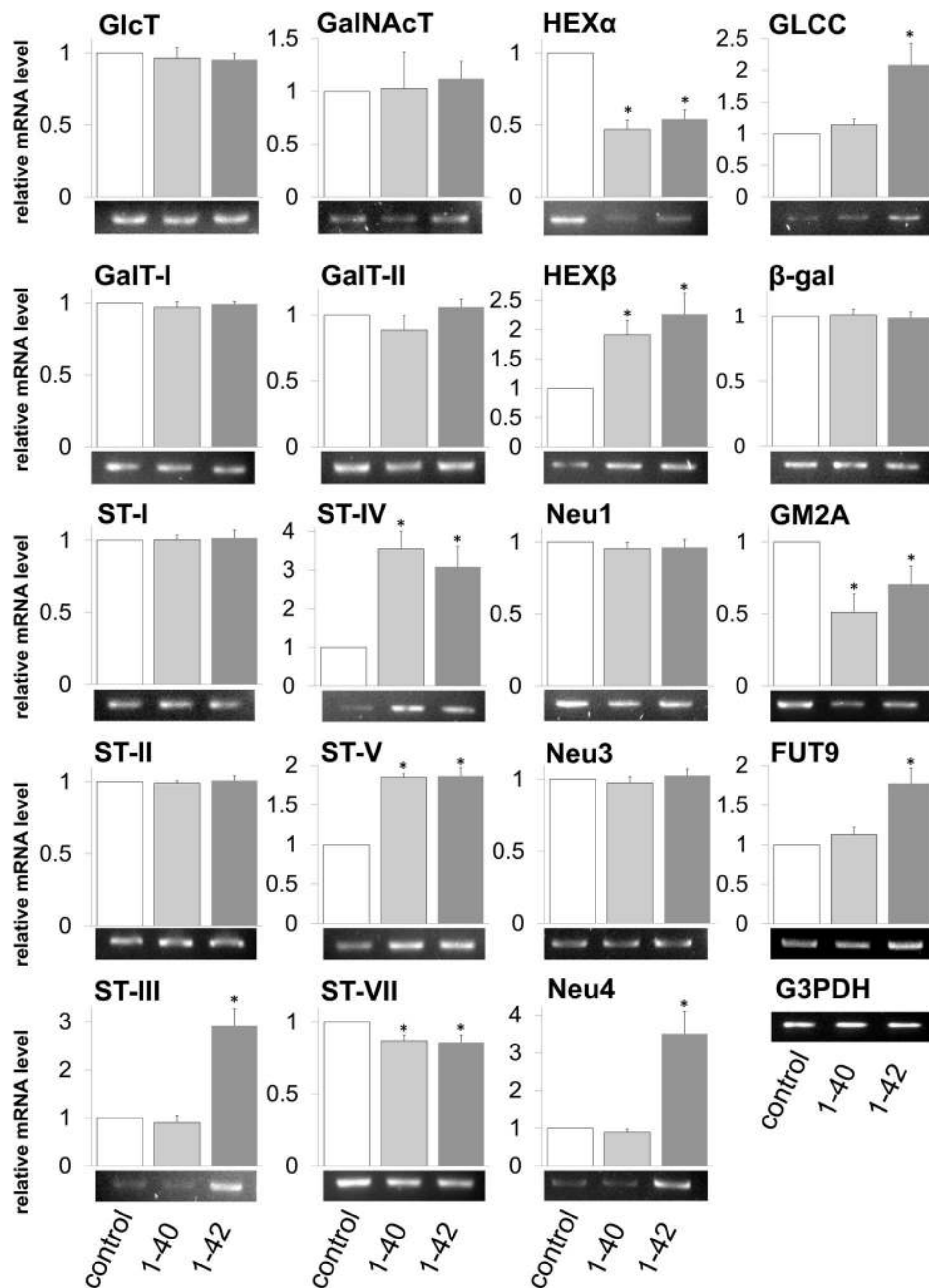

Fig. 3. Aβ treatment alters glycosyltransferases and glycosidases expression in NSCs

NSCs were cultured for 3 days with 10 μM of Aβ1-40 or Aβ1-42, or without Aβs. RT-PCR analyses were performed using specific primer sets. The enzymes for ganglioside metabolism are shown in Fig.1. Neu1, lysosomal sialidase; Neu3, plasma membrane sialidase; Neu4; mitochondrial sialidase; FUT9, α1–3 fucosyltransferase IX. G3PDH was used as a control. RT-PCR products were resolved on agarose gels and the intensity was quantified with normalization against G3PDH mRNA. The normalized value from control (vehicle) is defined as 1.0. Each bar represents mean ± SD of 3 independent experiments (n

= 3). * (p<0.05) indicate the level of significance in two tailed t-tests of differences between vehicle versus each treatment.

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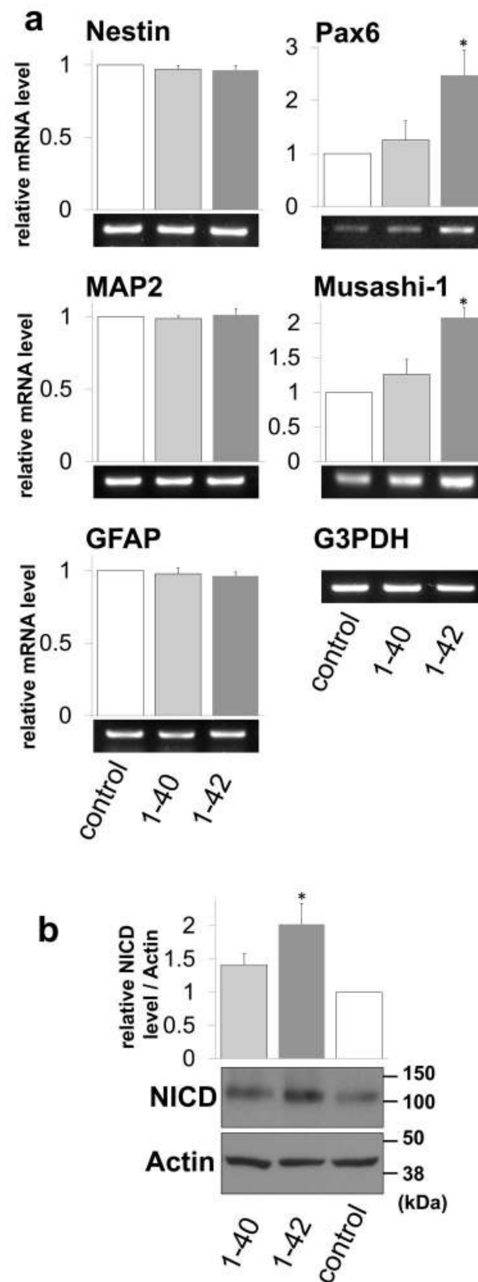

Fig. 4. Expressions of neural cell lineage-associated markers

NSCs were cultured for 3 days with 10 μM of Aβ1-40 or Aβ1-42, or without Aβs. **a** RT-PCR analyses were performed for Nestin, MAP2, GFAP, Pax6 and Musashi-1. RT-PCR products were resolved on agarose gels and intensity was quantified with normalization against G3PDH mRNA. The normalized value from control (vehicle) is defined as 1.0. **b** Western blot of NSCs treated with Aβs, and immunostained using anti-Notch1 intracellular domain (NICD), and anti-actin antibodies. Values were normalized by setting the NICD/Actin protein ratio for control (vehicle) in each treatment. Each bar represents mean ± SD of 3

independent experiments (n = 3). * ($p<0.05$) indicate the level of significance in two tailed t-tests of differences between vehicle versus each treatment.

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Table 1

Primer sequences used in this study

Abbreviation	Enzyme name	Sequence of primer
GlcT	GlcCer-synthase; Ceramide glucosyltransferase	5′-TGCATTTCATGTCCATCATCTAC-3′ 5′-GTCATCTGATTCACCATGTCAG-3′
GalT-I	Laccer-synthase; UDP-Gal, GlcCer β1-4 galactosyltransferase	5′-TCTACTTCATCTATGTGGCTCC-3′ 5′-AGAAGAGCTGATGGACTTCATC-3′
ST-I (ST3Gal-V)	GM3-synthase; CMP-NeuAc, Laccer α2–3 sialyltransferase	5′-TTTGGAGTCTGGCTCCTGTAC-3′ 5′-CTCTCAAGTGTTCAGGAAAGTC-3′
ST-II (ST8Sia-I)	GD3-synthase; CMP-NeuAc, GM3 α2–8 sialyltransferase	5′-ATGCTAGCTCGGAAATTCCCG-3′ 5′-CAGGGTCACAGCAGTCTTCC-3′
ST-III (ST8Sia-III)	GT3-synthase; CMP-NeuAc, GD3 α2–8 sialyltransferase	5′-TCTTCACCACTCCCAAGTACG-3¢′ 5′-CTGACTCCCTGTCAAGATTCC-3′
GalNAcT	GA2/GM2/GD2/GT2-synthase; UDP-GalNAc, LacCer/GM3/ GD3/GT3 β1–4 N-acetylgalactosaminyltransferase	5′-ATCAAGGAGCAAGTGGTGGAG-3′ 5′-CTATCAGCAGCTGGTCAGCC-3′
GalT-II	GA1/GM1/GD1b/GT1c-synthase; UDP-Gal, GA2/ GM2/GD2/GT2 β1–3 galactosaminyltransferase	5′-TTGATTTCTAACTCTCATGCCTG-3′ 5′-TCTTTGTATCAGCTCTGACACC-3′
ST-IV (ST3Gal-II)	GM1b/GD1a/GT1b/GQ1c-synthase; CMP-NeuAc, GA1/GM1/ GD1b/GT1c α2–3 sialyltransferase	5′-CATGGCTACCTTGCCCTACC-3′ 5′-CCAGGCACGATCTGGAACAG-3′
ST-V/III (ST8Sia-V)	GD1c/GT1a/GQ1b/GT3-synthase; CMP-NeuAc, GM1b/GD1a/ GT1b/GD3 a2–8 sialyltransferase	5′-AAGGAGATCAACAGCGCTGAC-3′ 5′-TACTGCGGGTGGAAGAAGTAG-3′
ST-VII (ST6GalNAc-VI)	GD1a/GT1aa/GQ1ba/GP1ca-synthase; CMP-NeuAc, GM1b/GD1a/ GT1b/GQ1c a2–6 sialyltransferase	5′-GCGGTCAGCAGTGTTTGTGAT-3′ 5′-AGCACACGGAATACACTGGAAT-3′
GLCC	Glucosylceramidase, Glucocerebrosidase	5′-CGGTATCTTGGGCATATGGTG-3′ 5′-GAAGTTGGATAACTGGAAGTCG-3′
HEXα	b-N-acetylhexosaminidase a-subunit	5′-TTCCAGTTCCGGTACCATGTC-3′ 5′-TTGTATGCCATGACATCCAGTG-3′
HEXβ	b-N-acetylhexosaminidase b-subunit	5′-TACAAGAGACATCATGGCCCTG-3′ 5′-ATCGTTTGGTGTATAGACATGAG-3′
Neu1	Lysosomal sialidase	5′-AAGTTCATCGCCATGAGGAGG-3 5′-TCGGGGTTGAAATCGTGATCG-3′
Neu3	Plasma membrane sialidase	5′-ACTGATGGAGGCCACATTACC-3′ 5′-TGAACTTGCCATGGTGCCATG-3′
Neu4	Mitochondrial sialidase	5′-AGCACTCTGGTACCATCTTCC-3′ 5′-AGAGGGCTTCGAGCATTACAG-3¢′
β-Gal	b-N-acetylhexosaminidase a-subunit	5′-TGATGTGGAGCATTTCATCCAG-3′ 5′-GTGTGATATTGTTGCCTGTTCC-3¢′
GM2A	GM2 activator protein	5′-ATGAAGGAAAGGACCCTGCAG-3′ 5′-GAGGCAGCAATCTTGATGCAG-3′
FUT9	fucosyltransferase-IX	5′-TTCGCCCATTTCTAATCGTC-3′ 5′-TTGTGCTCACCGTCAAGAAG-3′
Nestin		5′-ATGGTGGAATGCAAGAAAGC-3 5′-ATACCCAGCATGAAGGCATC-3′
MAP2		5′-CCTCAGCTGACAGAGAAACAG-3′ 5′-CTTGGTTCTGTGCTCTGTTTT C-3′
GFAP		5′-ATCGAGATCGCCACCTACAG-3′ 5′-CTCACATCACCACGTCCTTG-3′
Pax6		5′-CTGTACCAACGATAACATACCC-3′ 5′-AGGAGTGTTGCTGGCCTGTC-3′
Musashi-1		5′-AGCCATGTACGTAGCCATCC-3′ 5′-TCTCAGCTGTGGTGGTGAAG-3′
G3PDH	Glyceraldehyde-3-phosphate dehydrogenase	5¢-ACCACAGTCCATGCCATCAC-3′ 5¢-TCCACCACCCTGTTGCTGTA-3′