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Research Article

Knockdown of BACE1-AS Nonprotein-Coding Transcript Modulates Beta-Amyloid-Related Hippocampal Neurogenesis

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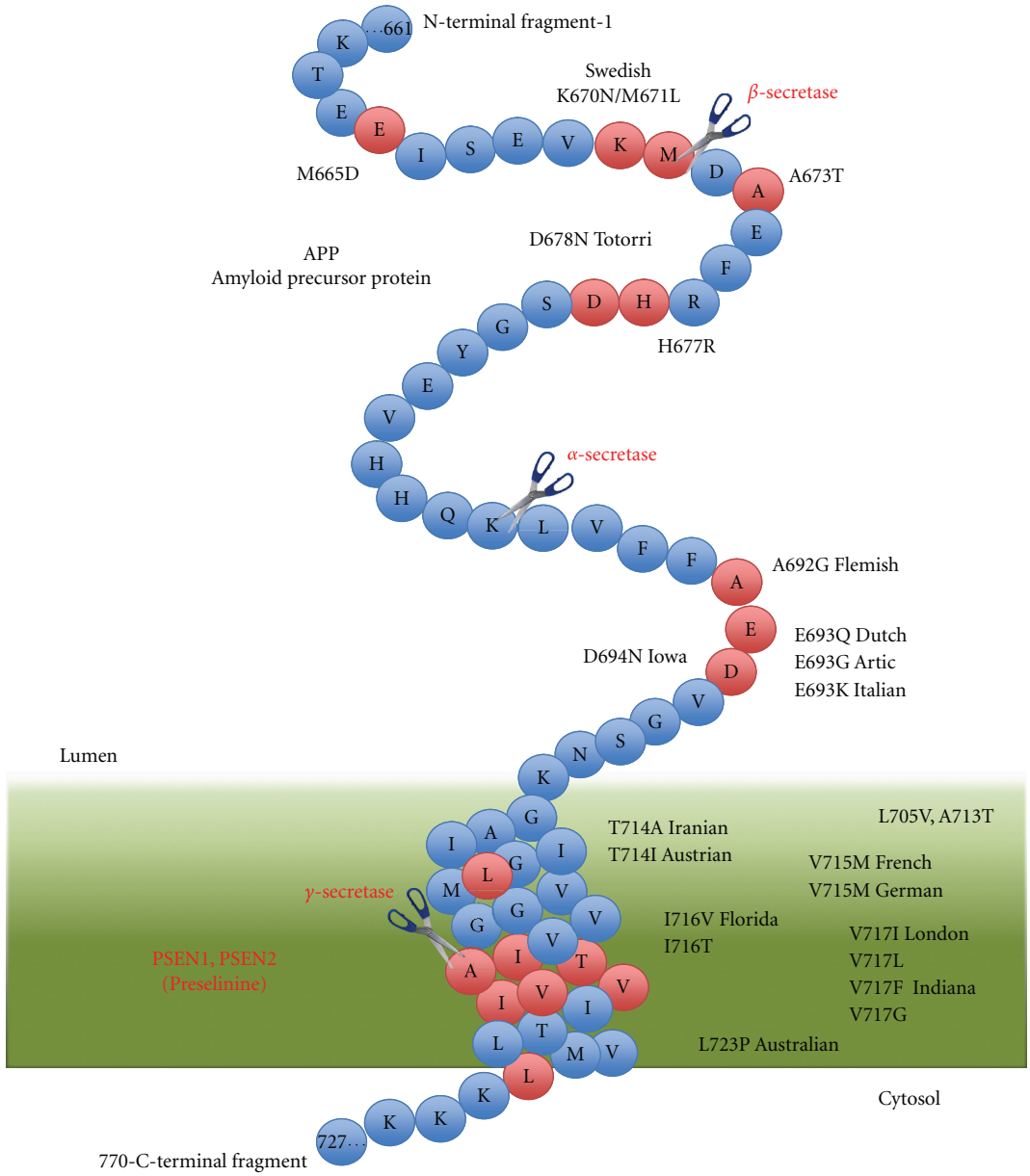
Background. Alzheimer's disease (AD) is a devastating neurological disorder and the main cause of dementia in the elderly population worldwide. Adult neurogenesis appears to be upregulated very early in AD pathogenesis in response to some specific aggregates of beta-amyloid (Aβ) peptides, exhausting the neuronal stem cell pools in the brain. Previously, we characterized a conserved nonprotein-coding antisense transcript for β-secretase-1 (BACE1), a critical enzyme in AD pathophysiology. We showed that the BACE1-antisense transcript (BACE1-AS) is markedly upregulated in brain samples from AD patients and promotes the stability of the (sense) BACE1 transcript. In the current paper, we examine the relationship between BACE1, BACE1-AS, adult neurogenesis markers, and amyloid plaque formation in amyloid precursor protein (APP) transgenic mice (Tg-19959) of various ages. *Results*. Consistent with previous publications in other APP overexpressing mouse models, we found adult neurogenesis markers to be noticeably upregulated in Tg-19959 mice very early in the development of the disease. Knockdown of either one of BACE1 or BACE1-AS transcripts by continuous infusion of locked nucleic acid- (LNA-) modified siRNAs into the third ventricle over the period of two weeks caused concordant downregulation of both transcripts in Tg-19959 mice. Downregulation of BACE1 mRNA was followed by reduction of BACE1 protein and insoluble Aβ. Modulation of BACE1 and BACE1-AS transcripts also altered oligomeric Aβ aggregation pattern, which was in turn associated with an increase in neurogenesis markers at the RNA and protein level. *Conclusion*. We found alterations in the RNA and protein concentrations of several adult neurogenesis markers, as well as non-protein-coding BACE1-AS transcripts, in parallel with the course of β-amyloid synthesis and aggregation in the brain of Tg15999 mice. In addition, by knocking down BACE1 or BACE1-AS (thereby reducing Aβ production and plaque deposition), we were able to modulate expression of these neurogenesis markers. Our findings suggest a distortion of adult neurogenesis that is associated with Aβ production very early in amyloid pathogenesis. We believe that these alterations, at the molecular level, could prove useful as novel therapeutic targets and/or as early biomarkers of AD.

1. Background

Alzheimer's disease is the most common form of dementia characterized by progressive impairment of cognition and short-term memory loss. The deposition of Aβ 1–42 in senile plaques is an established feature of AD neuropathology. Different species of Aβ have been shown to have toxic effects *in vitro* and *in vivo*; however, controversy still exists about whether the amyloidogenic pathway is the initiating mechanism for AD pathogenesis, or a mere consequence of other pathogenic events. Several human amyloid precursor protein (APP) mutations, as well as mutations in Presenilin-1 and Presenilin-2 were identified as genetic causes of familial AD [1] (Figure 1). These mutations related to familial and



A: alanine; D: aspartic acid; E: glutamic acid; F: phenylalanine; G: glycine; H: histidine; I: isoleucine; K: lysine; L: leucine; M: methionine; N: asparagine; P: proline; Q: glutamine; R: arginine; S: serine; T: threonine; V: valine; Y: tyrosine.
Aβ 1–42 sequence: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
Aβ 1–40 sequence: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Figure 1: Amyloid precursor protein (APP) cleavage and mutation. The present diagram represent APP, a transmembrane protein precursor of Aβ peptide, with the three principal cleavage site (β-secretase or BACE, α-secretase, and γ-secretase) and several known mutations.

early onset AD formed the basis for the amyloid cascade hypothesis to explain the pathogenesis of AD. β-secretase (BACE1) is the rate-limiting enzyme responsible for the production of Aβ peptides. Oligomeric forms of Aβ 1–42 can induce other components of AD pathophysiology, such as mitochondrial dysfunction [2, 3], Tau hyperphosphorylation (Tau hypothesis) [4], NMDAR endocytosis (channel hypothesis), excessive calcium influx (calcium hypothesis) [5, 6], synaptic dysfunction [7], neuronal stress [8], apoptosis (neurovascular hypothesis) [9], and aberrant neurogenesis [10, 11]. Therefore, BACE1 seems to be a critical component of many pathologic pathways, as its dysregulation can lead to excessive production of Aβ 1–42, formation of oligomeric Aβ species, and development of amyloid plaques.

Pluripotent neural stem cells (NSC) are present in the embryonic as well as adult brain [12–16]. Neural stem cells can be isolated from various parts of the brain and expanded *in vitro*, in the presence of mitogenic factors such

as epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) [17]. However, NSC remain quiescent, or are able to differentiate only to glial cells, in most parts of the adult nervous system with the exception of two regions, defined as neurogenic niches, where new neurons are actively generated, *in vivo*. Neurogenic niches, comprising the subventricular zone (SVZ) and subgranular zone of the hippocampus, contain active stem cells that differentiate towards neurons in the postnatal mammalian brain throughout their life under normal physiologic conditions [18, 19]. In the neurogenic niches, NSC are able to self-renew, proliferate, differentiate towards neurons, and incorporate into the neuronal circuitry [20]. Some reports indicate age-related decreases in adult neurogenesis that might be associated with a decline in hippocampus-dependent learning [18, 19, 21–23]. As memory loss and impaired learning are two of the most pronounced behavioural symptoms of AD, it seems likely that changes in neurogenesis could play a role in the progression of the disease. Increased neurogenesis has been described in neuronal stem cells treated with Aβ peptides *in vitro* [10], in animal models of AD [11] and in AD patients [24]. Interestingly, in the J20 APP transgenic mouse model of AD the observed *in vivo* effect of Aβ peptides on neurogenesis is mainly age dependent. In J20 mice, hippocampal neurogenesis is greatly enhanced at a young age, long before manifestation of any other AD-related symptoms, and dramatically reduced in older animals, when amyloid plaques are present and cognitive impairment is apparent [11]. Therefore, adult neurogenesis seems to correlate with the production of Aβ peptides and the accumulation of specific aggregates of Aβ in the brain. We sought to determine whether another mouse model of AD (Tg-19959) exhibits alterations in adult hippocampal neurogenesis markers and if so, to relate these changes with the levels of Aβ production and aggregation. Lastly, sought to determine whether we could modify these changes by targeting non-protein-coding RNAs.

Non-protein-coding RNAs (ncRNAs) appear to play an instrumental, but largely abandoned, role in human complex disorders [25]. Natural antisense transcripts are a group of regulatory RNAs, transcribed from the opposite strand of other (sense) genes [26]. Antisense transcripts are mainly ncRNAs and have been reported to regulate gene expression, either positively or negatively, through various mechanisms [27]. The discovery of BACE1 as the rate-limiting enzyme in the production of Aβ peptides has made it a prime therapeutic target for AD. Recently, we have identified and characterized one sense-antisense pair, BACE1 and its antisense partner BACE1-AS, and demonstrated a critical role of this non-protein-coding natural antisense transcript in AD [28]. BACE1-AS has the potential to participate in an ncRNA network that serves to fine-tune BACE1 protein output in the nervous system.

In the current study, we investigated mechanisms behind Aβ-induced hippocampal neurogenesis in the Tg-19959 model of AD. In particular, we investigated the extended effects of BACE1-AS transcripts on amyloid production, aggregation and plaque formation and their effects in adult neurogenesis *in vivo*.

2. Results and Discussion

2.1. Neurogenesis over Time in Tg-19959 Mice. We assessed adult neurogenesis in hippocampus at different time points throughout the life of Tg-19959 mice. We measured cell proliferation (by Ki67 staining) in the dentate gyrus of wildtype and Tg-19959 mice at different ages and observed a significant increase in NSC proliferation in young Tg-19959 animals (5 weeks old), as compared to age-matched wildtype mice (Figures 2(a) and 2(b)). This enhanced proliferation of NSC persisted over time as the animals aged (Figure 2(c)). We also quantified neural stem cell and progenitor markers in these mice over time, both at the RNA and protein level by RT-PCR and Western blot, respectively. Nestin is an intermediate filament protein used as a marker of pluripotent neural progenitors. It is expressed in type 1 and 2 cells in the adult hippocampus and it would represent the amount of neural progenitors and early neuronal progenitors in adult neurogenesis. Doublecortin (DCX) is a microtubule-associated protein expressed in neuronal committed precursor cells and immature neurons (type 2 and 3 cells) in the adult hippocampus [29]. The quantification of nestin and doublecortin levels in the hippocampus indicated a significant increase of both markers in Tg-19959, as compared to wild type mice both at the RNA (Figure 2(d)) and protein level (Figure 2(e)). Increased levels of neurogenesis markers were maintained over time as the animals aged. These data are consistent with the increased proliferation observed by Ki67 staining in the dentate gyrus and indicate that there is an early and persistent enhancement of hippocampal neurogenesis in Tg-19959 mice versus age-matched wild type mice.

2.2. Aβ Accumulation over Time in Tg-19959 Mice. Tg-19959 mice exhibit detectable Aβ plaques as early as 2 months of age and soluble Aβ peptides are detectable by 4 weeks of age (Figure 3). Five regions of Tg-19959 mouse brain (prefrontal cortex (PFC), hippocampus anterior (HPA), hippocampus posterior (HPP), striatum (ST), and cerebellum (CB)) were isolated at different ages and the levels of Aβ 1–42 and Aβ 1–40 quantified by AlphaLISA. Transgenic mice showed an age-dependent accumulation of soluble Aβ 1–40 and Aβ 1–42 in all regions examined (Figures 3(a) and 3(b)). Western blot analysis of hippocampal lysates from these mice showed an age-dependent accumulation of soluble Aβ aggregates (Figure 3(c)). Immunohistochemistry using 48G antibody, show a time-dependent increase in insoluble deposits of Aβ in hippocampus (Figures 3(d)–3(h)).

We hypothesized that specific soluble oligomeric Aβ aggregates might be involved in enhancement of neurogenesis over time, as we explained in a previous review [30]. Numerous reports have shown the important role of oligomeric Aβ species in AD pathologic processes [31–34]. In our studies, we show that Tg-19959 mice display an age-dependent enrichment of oligomeric Aβ species (Figure 3(c)). Our data show that compared to age-matched wild type mice, neurogenesis is greatly increased in Tg-19959 mice as early as 5 weeks of age, and at all subsequent ages examined. This increase in neurogenesis occurs in very early ages, correlates with detectable levels of Aβ aggregates in

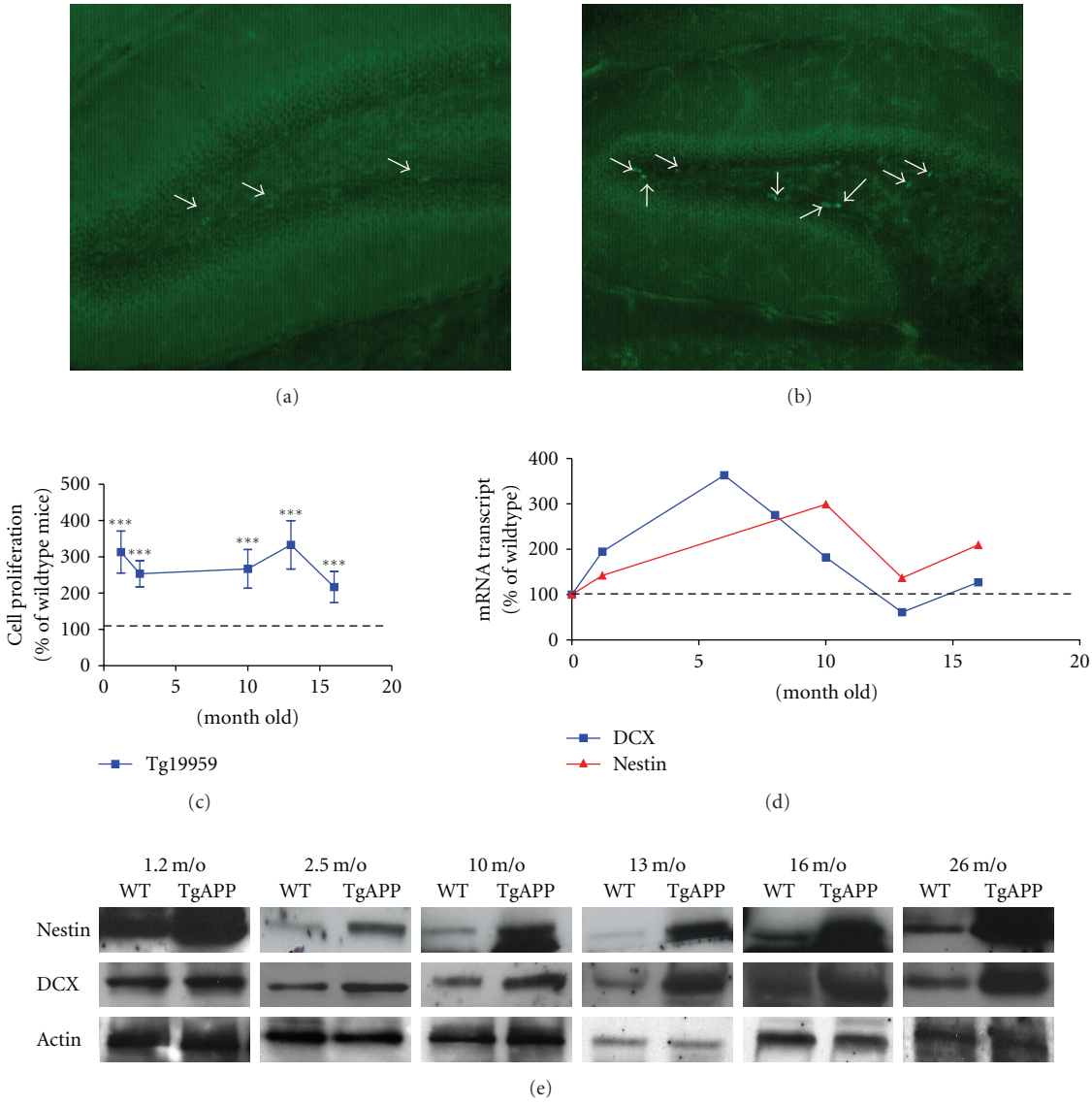


Figure 2: Tg-19959 mice exhibit increased proliferation in the dentate gyrus at all ages. We measured Ki67, a marker of cell proliferation in the dentate gyrus of wild type and Tg-19959 mice at different ages. There is significant increase in NSC proliferation in 5-week-old Tg-19959 mice (b) versus littermates wild type (a) that is maintained over time as animals aged (c). The markers of nestin (neural progenitor marker) and doublecortin (DCX, neuronal progenitor marker) were also significantly increased versus wild type in both mRNA (d) and protein (e) levels in hippocampus at all ages.

the brain, and it precedes any cognitive deficits in the mice, suggesting a relationship between Aβ and neurogenesis. These results also agree with our previous findings, where we described that specific oligomeric Aβ species are able to induce neurogenesis *in vitro* [10].

Consistently with our present results, in J20 APP overexpressing mice, there is a significant NSC proliferation in 3-month-old animals versus wild type animals. However, the neurogenesis rate in J20 mice decreases below wild type levels in older mice [11]. In Tg-19959 mice, we observed that this increase in neurogenesis in younger animals is more persistent and it extends to the aged animals. It has been reported that different aggregation states can play an important role in the effects of Aβ in cell death and/or neurogenesis [10, 32]. In addition, we showed an increased neurogenesis in Tg-19959 mice that is potentially related to enrichment of particular oligomeric assemblies [30]. We hypothesize that the differences in Aβ production and subsequent aggregation between both animal models could explain these differences. For this reason, the possibility to regulate Aβ production by manipulating BACE1 activity could open many possibilities in the neurogenesis-AD field.

2.3. Knock-down of BACE1-AS and BACE1 Transcript In Vitro. The modulation of BACE1 by ncRNAs could be a good tool to modify Aβ production, its further aggregation, and

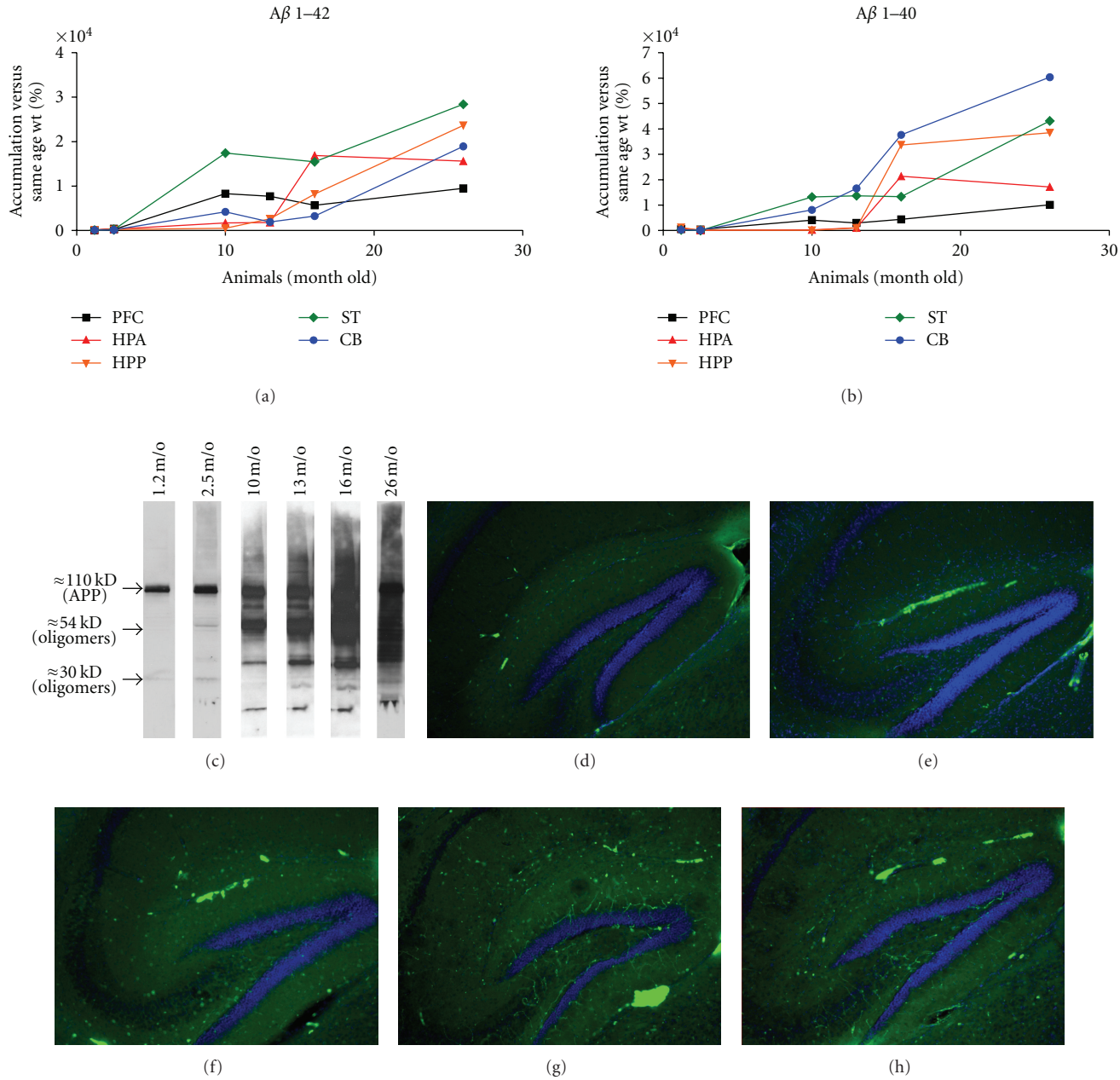


Figure 3: β-Amyloid peptide accumulates with aging in Tg-19959 APP transgenic mice. The level of soluble Aβ 1–42 and Aβ 1–40 amyloid peptides in prefrontal cortex (PFC), hippocampus anterior (HPA), hippocampus posterior (HPP), striatum (ST), and cerebellum (CB) at different ages, compared to wild type controls were quantified by AlphaLISA. Both Aβ 1–42 (a) and Aβ 1–40 (b) were gradually increased as animals aged, in all regions studied. The Western blot of hippocampal samples at different ages using the 48G antibody shows an increase over time in specific aggregation of Aβ (c). Immunohistochemistry with the 4G8 antibody against Aβ at different ages shows insoluble Aβ deposits in Tg-19959 hippocampi as early as in 2-month-old animals. (d): 2 m/o, (e); 6 m/o, (f): 10 m/o, (g): 13 m/o, and (h): 26 m/o. Green: 4G8. Blue: Hoechst.

its effect in hippocampal adult neurogenesis. To assess the regulatory effects of BACE1-AS on BACE1 transcripts, we designed LNA-modified oligos for knockdown of BACE1 and BACE1-AS transcripts. We also designed LNA-modified oligos simultaneously targeting both BACE1 and BACE1-AS transcripts, with equal nucleotide composition on both sides. We learned from previously published work that the two strands of an siRNA duplex are not equally eligible for assembly into RNA-induced silencing complex (RISC) [35]. Asymmetric stability of the 5′ end of an siRNA duplex determines the degree to which each strand participates in the RNAi pathway. Therefore, we designed our siRNA targeting the overlapping region between BACE1 and BACE1-AS transcripts, with symmetric stability at both ends to

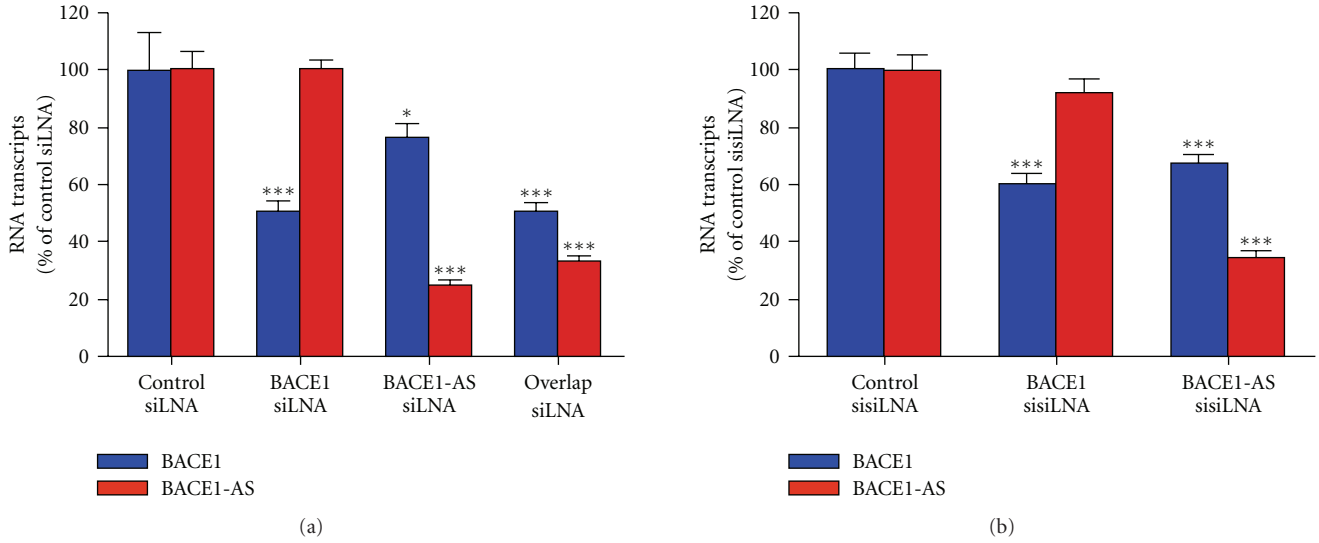


Figure 4: Effects of siLNA and sisiLNA in BACE1 and BACE1-AS RNA levels *in vitro*. To test the ability of siLNAs (a) and sisiLNAs (b) on BACE1 and BACE1-AS, we performed a study in mouse neuroblastoma N2a cells *in vitro*. Selective targets against BACE1-AS transcript were able to significantly knockdown not only BACE1-AS but also BACE1, supporting the proposed concordant regulation of BACE1 mRNA by BACE1-AS.

facilitate incorporation of both strands into RISC, resulting in simultaneous knockdown of both BACE1 and BACE1-AS transcripts. Additionally, we designed small internally segmented interfering RNAs (sisiRNAs) [36] for both BACE1 and BACE1-AS transcripts to be able to assess strand-specific effects of siRNA molecules. We examined LNA-modified oligos in mouse neuroblastoma N2a cells. In line with our previous findings [28], we found that selective targeting of the BACE1-AS transcript resulted in a statistically significant knockdown of not only the targeted BACE1-AS transcript, but also BACE1 mRNA (Figures 4(a) and 4(b)). Overlapping LNA modified siRNA reduced BACE1 and BACE1-AS transcripts, simultaneously. Strand-selective knockdown of BACE1-AS transcript, using sisiRNAs reduced the level of both BACE1 and BACE1-AS transcript (Figure 4(b)). These data confirm the proposed concordant regulation of BACE1 mRNA by non-protein-coding BACE1-AS transcript, in which the antisense transcripts change the level of the sense RNA or the corresponding protein abundance in a positive way.

2.4. Knockdown of BACE1-AS Reduces BACE1 Levels In Vivo. Having already demonstrated a role for BACE1-AS in regulating BACE1 function *in vitro*, we next assessed whether LNA-modified siRNAs show similar regulatory effects *in vivo* in mouse brain. We surgically implanted osmotic minipumps in the third ventricle of Tg-19959 mice and infused LNA-modified siRNA (1 mM) targeted to BACE1, BACE1-AS, the overlapping region between BACE1 and BACE1-AS transcripts, or an inert LNA-modified siRNA, as control (five animals per group). Continuous infusion of LNA modified siRNAs, over 14 days, resulted in reduced BACE1 mRNA levels across forebrain regions located adjacent to the third ventricle (Figure 5). Of the three siRNAs used, the overlapping siRNAs were most potently able to reduce BACE1 mRNA levels (approximately 60% knockdown of BACE1 in each of the brain regions examined), suggesting simultaneous targeting of both transcripts as the most potent therapeutic approach for reduction of BACE1, *in vivo* (Figures 5(a)–5(c)). Similar effect was found in BACE1 protein (Figure 5(d)). Surprisingly, we observed only a minimal knockdown of BACE1 after BACE1 siRNA infusion. This may be due to either a blockage of the osmotic mini-pump tubing, or due to an insufficient dose of siRNA since we have used a relatively low concentration of siRNA for these experiments (1 mM).

2.5. Assessment of Aβ Aggregation Species after BACE1 and BACE1-AS Knockdown. The ability to modulate Aβ production and its further aggregation could open new possibilities for AD therapeutics in relation to neurogenesis. We attempted to investigate whether we could alter Aβ accumulation by using siLNAs to BACE1, BACE1-AS, and the overlap region. Our initial *in vitro* experiments showed that siLNA targeting the BACE1 sense/antisense overlap region gave the greatest knockdown of both BACE1 and BACE1-AS mRNA, supporting our previous publication showing concordant regulation of BACE1 and BACE1-AS (Figure 4) [28]. Following 14 days of *in vivo* treatment with BACE1 sense/antisense overlap siLNA on Tg-19959 mice, we observed knockdown of BACE1 sense and antisense transcripts in all brain regions examined, confirming our *in vitro* data (Figure 5).

In order to determine whether the knockdown of BACE1 or BACE1-AS can alter the accumulation of particular oligomeric aggregates of Aβ, we examined Aβ aggregation in the hippocampus of mice after LNA-modified siRNA treatments. We homogenized mouse hippocampi after 14 days

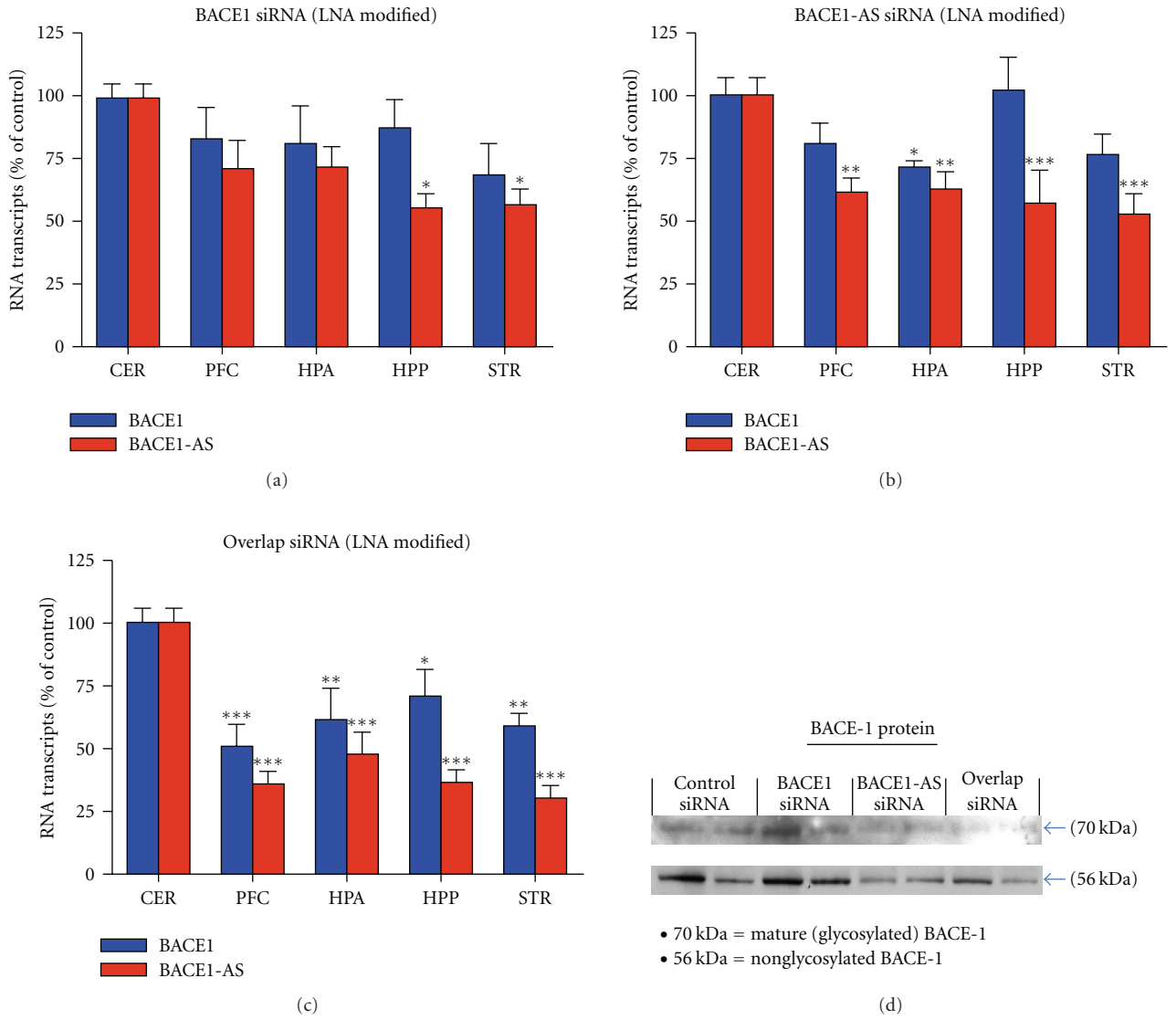


Figure 5: Effects of siLNA in BACE1 and BACE1-AS RNAs levels *in vivo*. To test the ability of siLNAs to affect BACE1 and BACE1-AS *in vivo*, we inserted a minipump as explained with overlap siLNA (a), BACE1-AS siLNA (b), and BACE1 siLNA (c) during two weeks. LNA-modified siRNAs reduced BACE1 mRNA levels in approximately 60% in each of the brain regions examined. In hippocampus, the protein levels of BACE1 were also clearly reduced (d, duplicates).

of LNA-modified siRNA infusion and measured Aβ 1–40 and 1–42, by alphaLISA. We found small in non-significant changes in the total amount of soluble Aβ (Figures 6(a) and 6(b)); however, we observed a significant reduction of insoluble (guanidine extracted) Aβ 1–40 and Aβ 1–42 levels after BACE-1-AS infusion (Figures 6(c) and 6(d)). Separation of Aβ species by Western blot revealed that all three LNA-modified siRNA treatments (BACE1 siLNA, BACE1-AS siLNA, and overlap siLNA) resulted in a significant change in Aβ aggregation pattern in mouse brain (Figure 6(e)). Several reports show the relation and equilibrium between soluble and insoluble levels of Aβ [37]. We did not detect significant changes in the total amount of soluble Aβ with the siRNA treatments; however, we found reduced levels of insoluble Aβ 1–40 and Aβ 1–42 (an indicator of plaque formation) in the brain regions examined (Figures 6(c) and 6(d)). Our data support our hypothesis that the modification of Aβ production is able to change the total amount of Aβ production by changing the pattern of aggregation. We hypothesize that a slower pace in Aβ production determines a slower aggregation that results in less accumulation in insoluble plaques.

Next, we assessed the effects of BACE1 and BACE1-AS reduction on DCX mRNA levels. We measured this marker of neuronal progenitors in the hippocampus after LNA-modified siRNA treatments. We found increased DCX mRNA levels in hippocampus (Figure 7(a)). We next measured the level of DCX protein in these same mice and

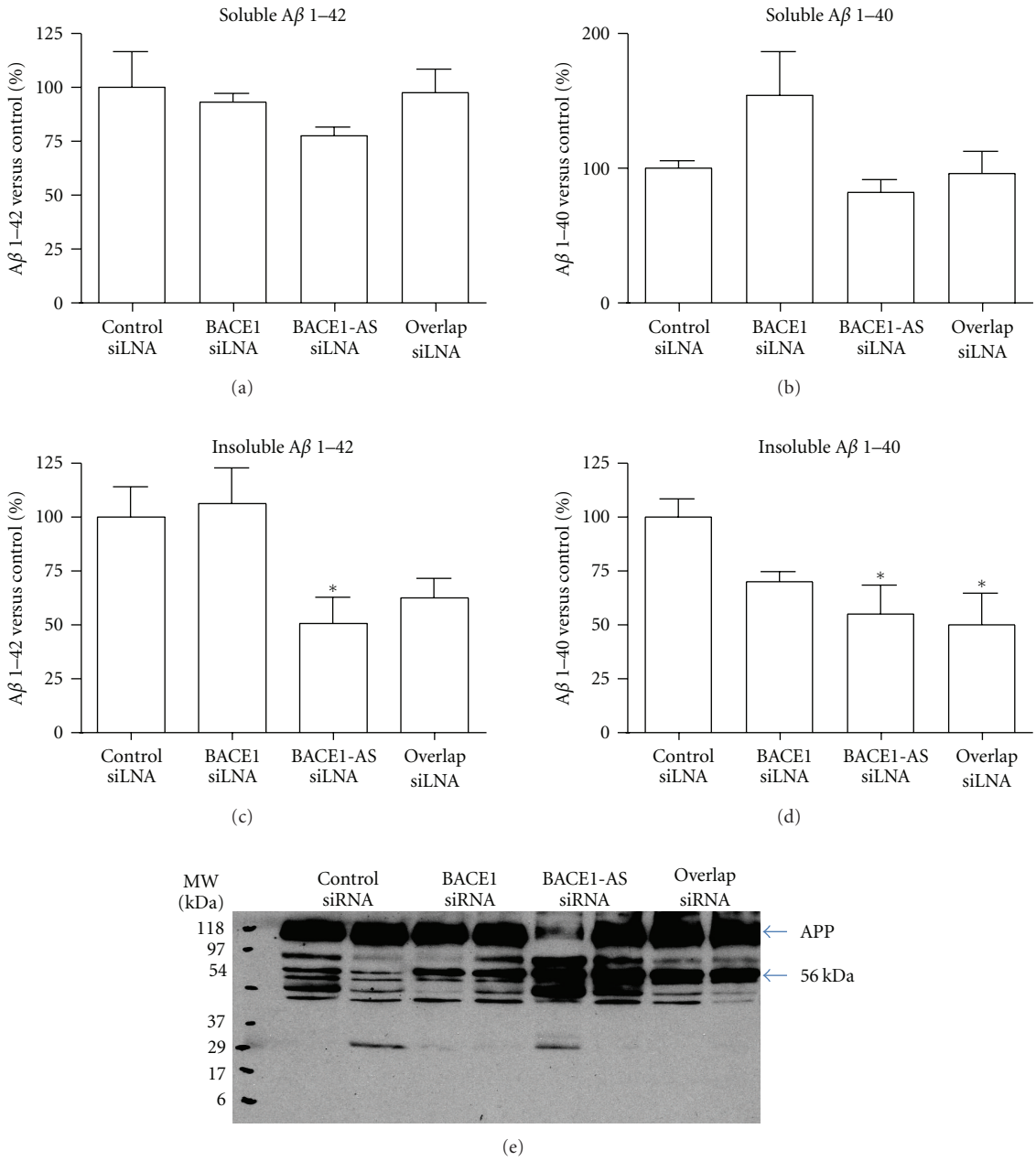


Figure 6: Effects of siLNA in for BACE1, BACE1-AS and overlap region in Aβ formation and aggregation. The infusion of siLNA for two weeks did not change the total amount of soluble Aβ in hippocampus (a), however, it did change the aggregation pattern (b). The measurement of insoluble Aβ (plaque aggregates) by guanidine extraction showed a significant decrease with BACE1-AS treatment (c-d).

observed an enhancement of DCX protein expression (Figure 7(b)). These data provide further evidence that particular Aβ assemblies may be responsible for driving neurogenesis in Tg-19959 mice, consistently with our previous reports in J20 mice [11]. We propose that the alteration of BACE1 protein levels changes the pace of Aβ production and ulterior aggregation state in APP overexpressing mice. This phenomenon would enrich the hippocampus in the soluble oligomers of Aβ responsible for the increased neurogenesis [10], inducing an increase in DCX. The final outcome would be the possibility of modulating adult neurogenesis in the AD brain by controlling BACE1 activity.

It is safe to predict that the observed increase in cell proliferation and neuronal differentiation in these AD animal models should result in an excess of neurons in the hippocampus. However, we did not detect morphological changes in cell number or tissue architecture in these mice. We postulate that there is an increase of newborn neurons in Tg-19959 mice; however, probably due to the lack of trophic factors or not reaching proper targets, most of these

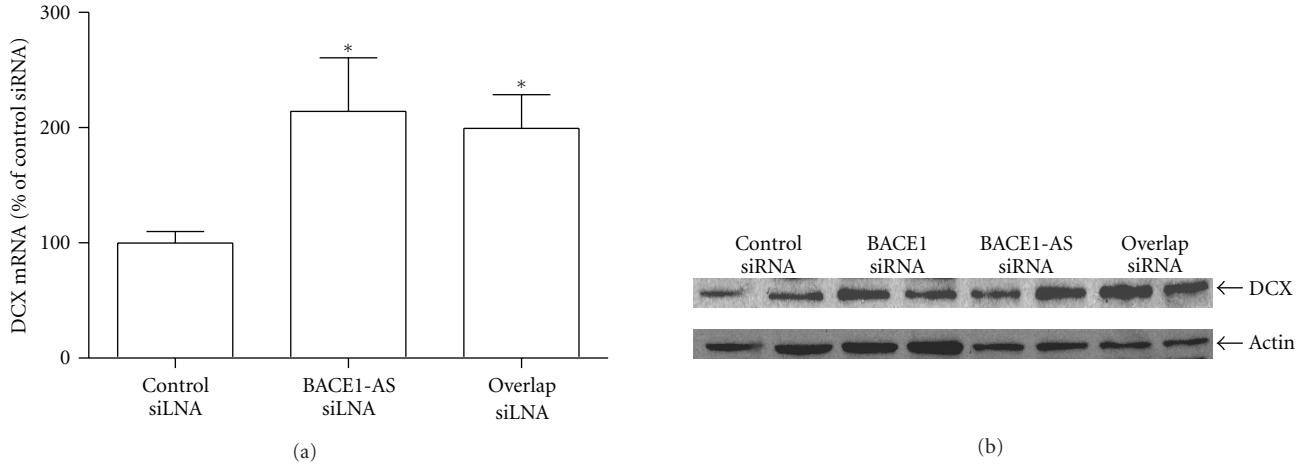


Figure 7: Effects of siLNA for BACE1, BACE1-AS, and overlap region in neurogenesis markers *in vivo*. The infusion of siLNA during two weeks *in vivo* increased the levels of the neuronal progenitor marker doublecortin (DCX) in both the mRNA (a) and protein (b, duplicates) level.

newborn neurons will probably die before reaching maturity. In our previous publication using J20 mice [11] we showed an increased neurogenesis (measured by Ki67 and BrdU) in younger animals and a decrease in older mice. We also describe that most of the newborn neurons died in one month, before reaching full maturity. We also speculate that it is possible that the overstimulation of NSC in early ages could have a negative effect in the NSC pool and neuronal turnover at older ages, contributing to a higher memory decline in older animals, as we suggested in a previous review [30]. The reason why we did not detect an increase in cell death in the mice is because the amount of newborn cells proliferation/death is very low versus the total number of cells, and a moderate increase in cell death could remain undetected. The final outcome of the Aβ-driven neurogenesis would not be a higher number of working neurons with Aβ production, quite the contrary, but we believe that the neurogenic response to Aβ and its manipulation can have a very important diagnostic value, because we could consider it the first symptom of AD, before cognitive impairment, cell death, and plaque formation, and the study of its mechanisms could be very useful in the development of new approaches in AD diagnostic and prevention.

3. Materials and Methods

3.1. Animal Surgeries. siLNAs were designed for mouse Bace1, Bace1-antisense, and overlapping region between two transcripts.

We selected 20 mice 8–12 weeks old, 5 animals per group for surgeries. We inserted cannula into the lateral ventricle of mice brain and connected the exit port to an Alzet micro osmotic pump model 1002. We prepared 100 nM of each siLNA, filled the micro pump, and infused it to the lateral ventricle of mouse brain, at rate of 0.25 μL per hour over a period of two weeks. Animals were sacrificed afterward and brain was extracted. Five brain regions were separated from each animal including cerebellum, prefrontal cortex, striatum, ventral hippocampus, and dorsal hippocampus. RNAs were isolated by TRIzol, and passed through RNeasy minicolumn (Qiagen) for on-column DNAse treatment. We made cDNA, using 400 ng of total RNA and random hexamers, followed by realtime PCR to assess the expression levels of target RNAs. Relative quantities were measured by normalizing ct values to β-Actin ct value.

3.2. Animal Studies. Experiments were performed following National Institute of Health guidelines and approved by The Animal Care and Use Committee (IACUC) at The Scripps Research Institute, Scripps, Florida. We are grateful to Dr. Leissring (Mayo Clinic) for providing APP transgenic (Tg-19959) mice, a model of AD that overexpresses a doubly mutated human APP [28]. We used Tg-19959 plus age- and sex-matched wild type littermates, at different ages for the *in vivo* experiments. The mice were sacrificed in CO_2 chamber followed by decapitation, and the brains were removed. Five tissues were dissected from each hemibrain; dorsal hippocampus, ventral hippocampus, cortex, dorsal striatum, and cerebellum and the other hemibrain were dissected for RNA and protein studies. The first hemibrain was fixed by immersion in 4% paraformaldehyde for 24 hours and washed several times in PBS for histology studies.

3.3. RNA Extraction and RT-PCR of the Mouse Brain Samples. We extracted RNA from dorsal hippocampus, ventral hippocampus, cortex, dorsal striatum, and cerebellum from each mouse brain, using Trizol reagent (Invitrogen), according to the manufacturer's protocol. RNA samples were passed through Qiagen RNeasy columns and were subjected to on-column DNAse treatment for removal of DNA contamination. We used 800 ng of each RNA sample for the first strand cDNA synthesis and carried out RT-PCR measurements. We plotted the percentile changes in RNA levels, for individual tissues compared to control mice, in each graph.

3.4. Realtime PCR (RT-PCR). We carried out RT-PCR with the GeneAmp 7900 machine (Applied Biosystems). The PCR reactions contained 20–40 ng cDNA, Universal Mastermix, 300 nM of forward and reverse primers, and 200 nM of probe in a final reaction volume of 15 μL. We designed the primers and probe using FileBuilder software (Applied Biosystems). They were strand-specific for sense-antisense pairs and the sense probes covered exon boundaries to eliminate the chance of genomic DNA amplification. The PCR conditions for all genes were as follows: 50°C for 2 min then 95°C for 10 min then 40 cycles of 95°C for 15 s and 60°C for 1 min. The results are based on cycle threshold (Ct) values. We calculated differences between the Ct values for experimental and reference genes (18 s RNA) as ΔΔCt and graphed as a percent of each RNA to the calibrator sample.

3.5. Western Blotting and ELISA. Brain tissues were homogenized in Tissue Extraction Reagent I (Biosource) containing protease inhibitor cocktail complete Mini (Roche) by 20 up-and-down passes in a glass Dounce homogenizer. Samples were centrifuged at 14,000 rpm for 30 min. Supernatants were used for ELISA quantification using an Aβ 1–40 and 1–42 AlphaLISA amyloid kit (PerkinElmer) and quantified with ENVISION 2104 Multilabel reader (PerkinElmer). Same supernatants were used for Western blot detection using mouse 4G8 antibody anti-Aβ (Signet), mouse Rat-401 anti-nestin (Developmental Studies Hybridoma Bank), and Rabbit anti-doublecortin (cell signaling).

3.6. Guanidine Extraction of Insoluble A-Beta Species. 200 μL of 6.25 M Guanidine-HCl (Sigma-Aldrich, MO) in 50 mM Tris at pH 8 was added to the pellet. The mixture was vortexed vigorously for 5 minutes until the pellet was fully dissolved. The solution was incubated overnight at room temperature. The following day, the mixture was centrifuged at 14,000 g for 10 minutes, and the supernatant collected into fresh tubes and labeled as Guanidine extracted (insoluble) fraction. Protein quantification and Aβ ELISA was performed as for the soluble fraction.

3.7. Immunohistochemistry. One hemibrain was fixed in phosphate-buffered 4% paraformaldehyde, pH 7.4, at 4°C for 24 hr, washed in phosphate buffer three times and embedded in 3% agarose for vibratome sectioning (Micron HM 650 V. Thermo Scientific). 50 μm sections of hemibrain were made in the sagittal axis. Sections were permeabilized in PBS with 1% TX-100 and maintained in this solution with 10% FCS with primary antibodies for two days with agitation. As a negative control, adjacent brain slices were similarly processed, except that the primary antibody was omitted. Rabbit anti Ki67 (Vector) and mouse 4G8 antibody anti-Aβ (Signet). Immunofluorescence secondary goat anti mouse or goat anti-rabbit Alexa Fluor 568 and 488 antibodies were used for detection.

4. Conclusions

We examined the pattern of neurogenesis in a transgenic mouse model of AD (Tg-19959) and found increased neurogenesis compared to age-matched wild type mice at all ages examined. This increase in neurogenesis occurs very early in the development of the disease, and is paralleled by an accumulation of Aβ oligomeric species. LNA-modified siRNAs targeting BACE1, BACE1-AS, or both transcripts reduced BACE1 at the mRNA and protein level, reduced the levels of insoluble Aβ peptides, and changed the aggregation pattern of soluble Aβ in the brain of Tg-19959 mice. Our data suggest that the increased neurogenesis observed in a mouse model of AD parallels the accumulation of oligomeric Aβ species and that we can manipulate it by using with LNA-modified siRNAs against BACE1 and BACE1-AS. We believe that the study of Aβ-driven neurogenesis could be a powerful tool in early detection of AD, and the use of ncRNAs could help to manipulate the development of the disease before the appearance of adverse symptoms.

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