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New Insights in Amyloid Beta Interactions with Human Telomerase

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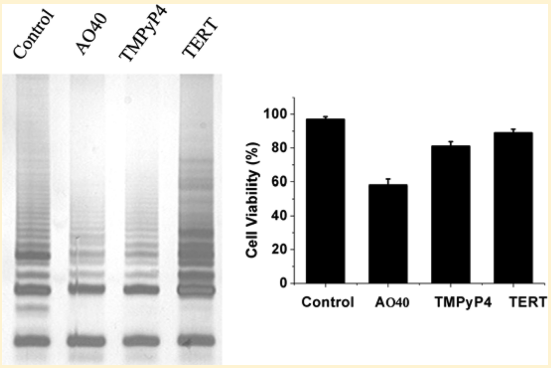
Supporting Information

ABSTRACT: It is well-known that aging is the most risk factor for Alzheimer's disease (AD). Recent studies have demonstrated that human telomerase is associated with pathological mechanisms of AD. In view of the central role of telomere and telomerase in the aging process, herein we found that the aggregated form Aβ (Aβ1-40 and Aβ1-42), not Aβ monomer, could inhibit telomerase activity both in vitro and in living cells. The β-sheet structures were essential for Aβ-induced telomerase inhibition. Further studies indicated Aβ oligomers inhibited telomerase activity through binding to DNA·RNA hybrid formed by telomeric DNA and the RNA template of telomerase, then blocking telomerase elongation of telomeric DNA. We also identified that intracellular Aβ localized at telomere, and induced cell senescence and telomere shortening. These results indicate that Aβ oligomers can be potential natural inhibitors of telomerase and that inhibition of telomerase activity may be one of the factors for Aβ-induced cytotoxicity. Our work may offer a new clue to a better understanding of aging and AD.



INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia characterized by amyloid plaques and neurofibrillary tangles.[1−3] The major constituent of amyloid plaques is the amyloid β-peptide (Aβ), which is prone to form β-sheet-rich structures like oligomers, protofibers, and fibers under physiological conditions.[4,5] The aggregated forms of Aβ are potent neurotoxins which are considered to play a critical role in the pathogenesis of AD.[6−8] Two main isoforms of Aβ, Aβ1-40 and Aβ1-42, have been known to be associated with AD. Though the conformational transition of Aβ from its native state to its fibrillation has been suggested to be a critical event in neuronal damage, the precise mechanism of Aβ pathogenesis in AD is still not clear.[9−15]

Telomerase is a ribonucleoprotein reverse transcriptase composed by a catalytic protein subunit (TERT) and an RNA template (TER). During cell division, telomerase catalyzes the addition of TTAGGG repeats to telomeric DNA to protect telomeres from erosion. Telomerase catalyzes the reaction by using its RNA template, which contains a partially redundant sequence complementary to TTAGGG.[16,17] Telomerase activity is highly associated with the cellular senescence and immortalization.[18] Recently, emerging evidence indicates that telomerase plays an important role in mammalian brain, even involves in the AD pathological process.[19−22] Upregulation of telomerase is proposed as a potential strategy for AD because overexpression of TERT can protect neurons against Aβ-induced apoptosis.[23−25] On the other hand, the TERT immunoreactivity was observed to decrease in Aβ treated neurons.[21,26,27] Telomere length of hippocampal neurons and T cells in patients with AD was significantly shorter compared with control subjects.[21,28,29] All these findings suggest that the antagonism relationship between telomerase and Aβ on neurons is viabile. However, the detailed mechanism underlying is not clear.

Here, we found that the aggregated form Aβ (Aβ1-40 and Aβ1-42) could inhibit telomerase activity in vitro and in living cells. We demonstrated the β-sheet structure of Aβ aggregates played an important role in the Aβ-induced inhibition of telomerase activity. The Aβ oligomers bound to telomeric DNA/RNA hybrids of telomerase, resulting in inhibition of telomerase activity. It was also demonstrated that Aβ oligomers had an anticancer effect as telomerase inhibitor. We also observed that intracellular Aβ localized at telomere and induced telomerase activity inhibition and telomere loss in cellular environment. Our results suggest that Aβ oligomers can be potential natural telomerase inhibitors.

RESULTS

We first investigated whether Aβ1-40 peptides could inhibit the telomerase activity in vitro. The Aβ peptides undergo structure transition during aggregation, leading to formation of soluble oligomeric intermediates, including oligomers, protofibrils, and finally the insoluble fibrils. To clarify the effect of the structure and morphology change of Aβ peptides on telomerase activity, we prepared Aβ monomers, oligomers, protofibrils, and fibrils, respectively, as described in the Materials and Methods. Results

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of Figure 1a showed the morphologies of various Aβ samples observed by atomic force microscopies (AFM). The monomer

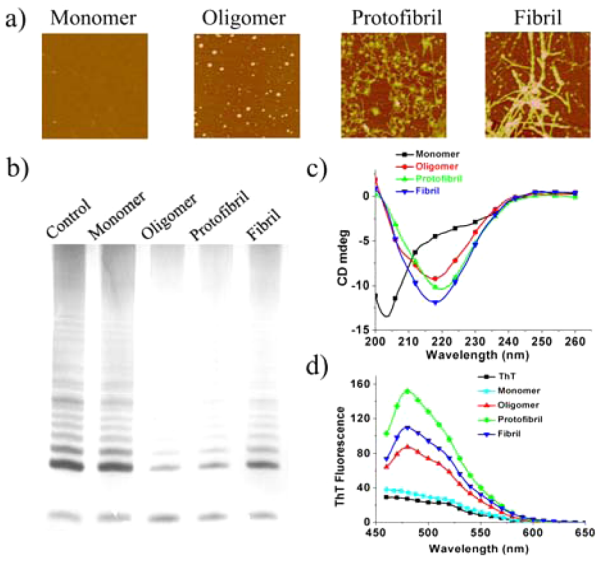

Figure 1. (a) AFM images of Aβ1-40 monomers, oligomers, protofibrils, and fibrils. Each image is 2 × 2 μm. (b) Aβ1-40 monomers, oligomers, protofibrils, and fibrils for telomerase activity inhibition were examined by TRAP assay. (c) CD spectra of Aβ monomers (black), oligomers (red), protofibrils (green), and fibrils (blue). (d) ThT fluorescence assay for Aβ monomers (cyan), oligomers (red), protofibrils (green), and fibrils (blue). The excitation wavelength was 444 nm.

was shown as little dot of 1 nm, while the oligomers were spherical with height from 1.5 to 6 nm. The protofibrils appeared as flexible rods of length up to 200 nm, at a height from 2 to 7 nm, with oligomers beaded. The height increased to 10 nm in fibrils, with a length of more than 1 μm. All the morphologies of the Aβ intermediates were consistent with previous reports.[30,31]

We next studied the telomerase inhibition effect of various Aβ samples. As shown in Figure 1b, telomerase inhibition was observed in all kinds of Aβ aggregates, except for Aβ monomers. The Aβ peptides undergo secondary structure transition from random-coil to β-sheet during aggregation process.[13] We therefore explored whether the inhibition of telomerase by Aβ peptides was β-sheet structure dependent. We characterized the secondary structure of Aβ aggregates by circular dichroism (CD), which was a widely used technique for studying Aβ conformations.[32−34] As shown in Figure 1c, the monomers had a negative peak near 200 nm, indicating the random coil structure. However, the Aβ aggregates had a wide band near 217 nm (Figure 1c), indicative of formation of β-sheet structure. We computed the proportional contribution of the different secondary structures to a given Aβ sample, revealing that the oligomerization was accompanied by an increase in β-sheet structure (Table S1).[35] This result was further supported by Thioflavin T (ThT) fluorescence assay (Figure 1d), which has been widely used to measure β-sheet structure formation.[36,37] These results indicated that telomerase inhibition could be related with β-sheet structure. It should be noted that telomerase inhibition capacity decreased in fibril sample compared to the protofibril, though the increased β-sheet structures. We ascribed this phenomenon to the poor water solubility because we observed precipitates in the fibril sample. These results implied the oligomeric β-sheet structures might be important for telomerase inhibition.

To further clarify this hypothesis, we prepared Aβ aggregates with or without the Aβ inhibitor, curcumin. Curcumin was a well-known Aβ binding ligand that can disrupt β-sheet structure and inhibit Aβ aggregation.[38−40] We used Aβ1-40 oligomers (AO40) as the aggregates in the following experiments unless otherwise described. Results of CD spectra and ThT fluorescence assay demonstrated that curcumin could successfully inhibit structural transition of Aβ from native random coil to β-sheet conformation (Figure S1a,b). Then, we evaluated the inhibition effect of AO40 prepared with curcumin on telomerase activity. As shown in Figure S1c, curcumin could effectively prevent the Aβ-induced telomerase activity inhibition. Control experiments were also carried out to demonstrate that curcumin alone had little effect on telomerase activity under our experimental conditions (Figure S1d). All these results indicated that β-sheet structures played an important role in Aβ-induced telomerase inhibition.

The IC_{50} of AO40 was about 0.48 μM under optimal conditions (Figure S2). Aβ1-42, another isoform of Aβ peptides, was more fibrillogenic and toxic, whose production was believed to be crucial in initiating amyloid formation and pathogenesis of AD.[41,42] We therefore prepared the Aβ1-42 oligomers (AO42) as described in the Materials and Methods. The telomerase inhibition effects were observed to be similar between AO40 and AO42 (Figure 2b), indicating that both of

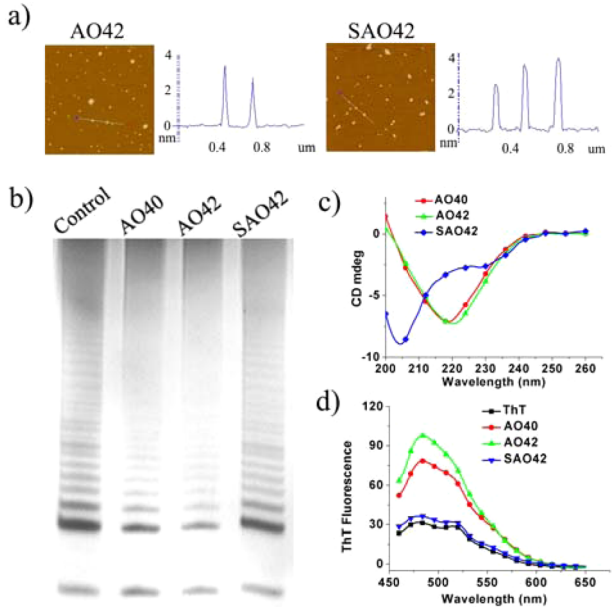

Figure 2. (a) AFM images of AO42 and SAO42. Each image is 2 × 2 μm. (b) TRAP assay for the telomerase inhibition effect of AO40, AO42, and SAO42. (c) CD spectra of AO40 (red), AO42 (green), and SAO42 (blue). (d) ThT fluorescence assay for AO40 (red), AO42 (green), and SAO42 (blue).

them could interact with telomerase. It is known that Aβ monomers form an oligomer through complex pathways, and the preparation method is essential for their conformation and toxicity.[43] It was reported that low temperature and lower Aβ concentrations could facilitate Aβ to keep in oligomer conformation.[44,45] To further clarify the relationship between telomerase inhibition effect and the conformation of Aβ peptides, we also prepared relatively stable Aβ1-42 oligomers

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(SAO42) according to previous report, which was considered to assemble in a fibril-independent pathway.[45] The morphologies of the AO42 and SAO42 were characterized by AFM, and the secondary structures were characterized by CD and ThT assay. As shown in Figure 2a, the height was similar between AO42 and SAO42. However, compared to the AO42, the SAO42 contained little β-sheet structures (Figure 2c,d), indicating that the SAO42 folded in a different way from the AO42. Correspondingly, the SAO42 had a much weaker telomerase inhibition effect than AO40 and AO42 (Figure 2b). We also characterized the aggregation state of the oligomers by using Tricine-SDS-PAGE. Multiple bands having trimer, tetramer, and higher order oligomers in AO40 and AO42 samples were observed, indicating the heterogeneity. In contrast, relative fewer bands of trimer and tetramer were observed in SAO42, implying its different aggregation pathway and conformer (Figure S3). The AO42 represents intermediates in the pathway of fibril formation and is highly dynamic. The SAO42 assembles in a fibril-independent pathway and therefore stays in the oligomer conformation with little β-sheet structures. These results further indicate that β-sheet structure is important for telomerase inhibition.

We further compared the oligomers of various peptide fragments of Aβ, such as 1-12, 12-28, and 25-35 for their inhibition effects on telomerase activity. As shown in Figure S4, the order of the inhibitory effect was, from highest to lowest, 25-35 > 12-28 > 1-40 > 1-12. It has been reported that some truncated variants of Aβ1-40 were more fibrillogenic.[46,47] Especially the Aβ25-35, which has been considered as the most fibrillogenic and toxic peptide fragment derived from Aβ1-40, showed the strongest telomerase inhibition effect.[47] In contrast, the Aβ1-12 peptide, which cannot form a β-sheet structure,[48] showed no inhibitory effect for telomerase activity. These results further supported our hypothesis that the β-sheet formation was essential for Aβ induced telomerase inhibition.

To gain the insight of the inhibitory mechanism of Aβ oligomers against telomerase activity, we carried out the recovery experiment. As shown in Figure S5a, excess telomerase could reverse the inhibition effect induced by AO40. This result suggested the Aβ oligomers might directly interact with telomerase to interrupt the enzyme activity. As mentioned in the Introduction, active telomerase consists of two components: the protein catalytic subunit TERT and the DNA/RNA hybrid duplex formed by template RNA and telomeric substrate DNA. To determine which part of telomerase interacts with Aβ, we used the telomerase extract treated by RNase (TETR, without RNA template) as the protein part and the hybrid formed between the RNA template, CUAACCCUAAC, and the substrate DNA, GTTAGGGTTAG as the nucleic acid part. We incubated 2 μM AO40 and 0.3 μg telomerase extract with excess hybrid or TETR. As shown in Figure S5a, the RNA/DNA hybrid could reverse the telomerase activity, but the TETR could not, indicating that Aβ could bind to the RNA·DNA hybrids (RDH) to inhibit telomerase activity. However, addition of excess calf thymus DNA (ctDNA) could not reverse the telomerase activity under the same conditions, suggesting that Aβ may recognize the RNA·DNA hybrids (RDH) of telomerase in a specific manner. To test this hypothesis, we further performed fluorescence titration experiments to compare the binding affinity (Figure S5b). The fluorescence intensity of Aβ was quenched much stronger with increasing the concentration of RDH than that of binding to ctDNA. The estimated binding constant of Aβ with RDH and ctDNA is about 5.96×10^5 and 3.82×10^4 M^{-1}, respectively, indicative of specific interaction between Aβ and RDH. Next we did further study on how Aβ interacted with RDH.

To characterize the interaction between Aβ oligomers and RDH, gel mobility shift assay was performed. AO40 was incubated with RDH, and the mixtures were analyzed on 10% PAGE gel. The gel was confirmed by silver staining. As shown in Figure 3a, AO40 apparently reduced the density of RDH,

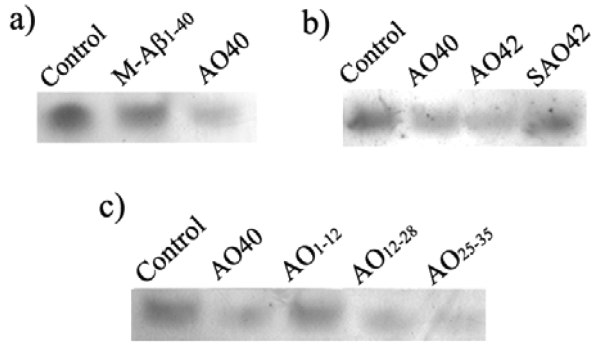

Figure 3. Characterization of the interaction between Aβ peptides and RDH. (a) RDH band intensity in the presence of Aβ1-40 monomer (M-Aβ1-40) or Aβ1-40 oligomers (AO40). (b) RDH band intensity in the presence of AO40, AO42, and SAO42. (c) RDH band intensity in the presence of Aβ peptide oligomers corresponding to residues 1-40, 1-12, 12-28, and 25-35. [RDH] = 5 μM; different [Aβ] = 5 μM. Experimental details are described in Materials and Methods section.

indicative of the interaction between the AO40 and RDH. In contrast, the Aβ monomers did not bind to RDH. Thermal denaturation studies were also carried out. Considering the large size of Aβ oligomers, when the AO40 bound to the RDH, it could unwind the duouble-stranded structure and destabilize the RDH. The T_m of RDH was decreased in the presence of AO40, however, in the presence of monomers, T_m did not change (Figure S6). This result further supported that aggregated Aβ, but not Aβ monomers, interacted with the RDH. The interactions led to the attenuated hydrogen bond between telomeric DNA and RNA template, which might be caused by the electrostatic repulsion between Aβ and phosphate group of nucleic acid because Aβ was negatively charged overall. These results suggested that the Aβ oligomers inhibited telomerase activity through binding to RDH.

The interactions of RDH with Aβ1-42 and other peptide fragments of Aβ were also compared by using gel mobility shift assay. As shown in Figure 3b, the interaction was similar between AO40 and AO42, which was much stronger than SAO42. Figure 3c showed that RDH band density was decreased following RDH bound to 25-35 > 12-28 > 1-40 > 1-12, which was consistent with the above telomerase inhibition data. This correlation further indicated that Aβ oligomers inhibited telomerase activity through binding to the DNA·RNA hybrid.

We and another group previously found that the aggregated Aβ interacted with DNA and induced DNA conformation transition.[48−51] Aβ oligomers binding to DNA in a DNA-sequence specific manner as a transcription factor has been recently reported, and an Aβ-interacting domain with a "G"-rich consensus of "KGGRKTGGGG" was determined.[52−54] The consecutive "G" sequence was demonstrated to play an important role in the Aβ-DNA specific interaction.[54] The RDH is also "G"-rich and has a consecutive "G" sequence.

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Therefore, it is plausible that Aβ oligomers bind to RDH in a specific manner and block telomerase activity.

The binding mode of Aβ oligomers bound to RDH was further studied. A commonly used competitive binding assay was carried out by using fluorescence and CD spectroscopy.[55−58] It is well-known that EB can intercalate into DNA through minor groove, and Hoechst 33258 is a classical DNA minor groove binder.[59] When bound to DNA, EB or Hoechst fluorescence is greatly enhanced. With this in mind, if the Aβ can competitively bind to the same sites to RDH as EB or Hoechst 33258, the fluorescence of EB or Hoechst 33258 would decrease because Aβ binding to RDH should exclude EB or Hoechst 33258 out of their binding sites. As shown in Figure S7, their fluorescence decreased about 20% upon the addition of the AO40, showing that Aβ competitively bind to the same sites. To further verify these results, another DNA binding agent, methylene green (MG), was used. MG is a proven DNA major groove binder.[60] When MG bound to RDH, three induced CD signals at 310, 620, and 650 nm, characteristic of MG bound to DNA, were observed (Figure S8). If Aβ binds to RDH major groove, they would exclude MG out of major groove and weaken MG-induced CD signals. As shown in Figure S8, the induced CD intensity was significantly decreased in the presence of AO40, suggesting that AO40 also bound to RDH in the major groove by replacement of MG molecules. In combination with fluorescence and CD competitive binding data, AO40 can bind to RDH in both major and minor groove. The specific DNA recognition by β-sheets peptides may have several modes.[61] It is well-known that the Aβ oligomers are partially homogeneous including dimmers, trimers, and oligomers, et al.[11] So, it is reasonable that Aβ oligomers bind to both the major and minor groove. Major groove may become the main binding site because the CD intensity decreases significantly. Besides, the major groove possesses more appropriate dimensions to match the Aβ oligomers.

The Aβ is negative charged overall under our experimental condition, and RDH is also negative charged. Therefore, electrostatic interaction is not the key factor for their interactions. We found that the oligomeric β-sheets structure played an important role in Aβ-induced telomerase activity inhibition. Therefore, hydrophobic interaction and peptide-DNA hydrogen bonds between the β-sheet and base pairs are important for their binding. The proposed recognition mode was shown in Figure S9 (PDB ID of telomerase: 3KYL and Aβ: 2BEG). The three-stranded β-sheets formed by Aβ may bind in the major groove of RDH via hydrophobic interaction. It should be admitted that the detailed structural information about their binding is not known. This requires much effort to achieve because telomerase is difficult to purify and Aβ aggregates can have diverse oligomers and structures.

We next investigated the correlation between the telomerase activity inhibition and the neurotoxicity for Aβ1-40 and Aβ1-42 oligomers. In agreement with previous study, the AO40, AO42, and SAO42 were all toxic to PC12 cells (Figure 4a). However, as studied in vitro, the SAO42 was much weaker than AO40 and AO42 on the telomerase activity inhibition in PC12 cells. For other peptide fragments of Aβ, the neurotoxicity was 25-35 > 12-28 > 1-40 > 1-12 (Figure S10a), which was consistent with the telomerase inhibition capacity in cells (Figure S10b). This order showed that the telomerase inhibition capacity correlated with the neurotoxicity for Aβ oligomers, except the SAO42 sample, which was lack of β-sheet structure. All these results indicated that the telomerase inhibition capacity of Aβ oligomers was β-sheet structure dependent. However, the factors influencing the cytotoxicity of Aβ oligomers might be different.

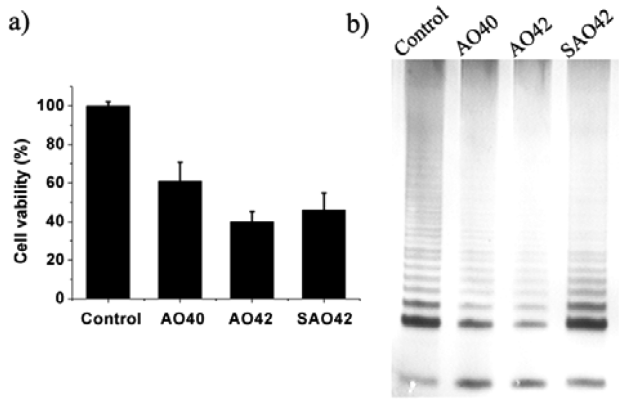

Figure 4. Cell viability and telomerase activity inhibition in PC12 cells after the treatment of AO40, AO42, and SAO42. (a) Cell viability was determined by using MTT method after the treatment of AO40, AO42, and SAO42. Data points shown are the mean values from three independent experiments. (b) Cells were treated by AO40, AO42, and SAO42, and the telomerase activities were examined by TRAP assay.

Since telomerase is overexpressed in most cancer cells, targeting telomerase has been considered as a specific anticancer therapy.[62,63] To this end, we studied the effect of Aβ oligomers on tumor cells. Human cervical cancer cells (HeLa) were treated with AO40 for 48 h, and the telomerase activity was evaluated by TRAP assay. A classical telomerase inhibitor, cationic porphyrin (TMPyP4),[64,65] was also used as a positive control. Figure 5a showed that AO40 dramatically decreased the telomerase activity in HeLa cells and was even

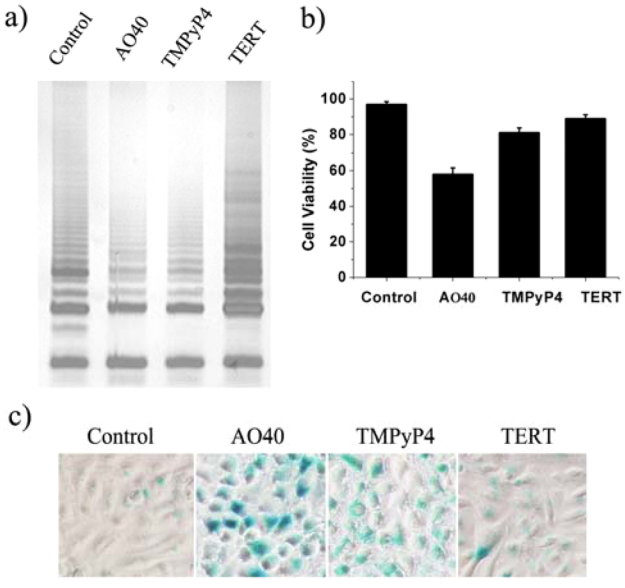

Figure 5. Effect of AO40 as telomerase inhibitor on HeLa cells. (a) TRAP assay for telomerase activity in HeLa cells treated with AO40, TMPyP4, or HeLa-TERT cells treated with AO40. (b) MTT assay for cell viability in HeLa cells treated with AO40, TMPyP4, or HeLa-TERT cells treated with AO40. (c) Expression of senescence-associated β-galactosidase (SA-β-gal) in HeLa cells treated with AO40, TMPyP4, or HeLa-TERT cells treated with AO40. Scale bar = 20 μm.

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comparable with TMPyP4. We also found the obvious occurrence of β-galactosidase activity in Aβ treated cells (Figure 5c), which was a marker of cellular senescence and was always associated with telomerase activity inhibition.[66] MTT assay demonstrated that AO40 induced rapid cell viability decease on HeLa cells (Figure 5b). The cellular senescence and reduction of cell viability induced by AO40 were even stronger than those induced by TMPyP4. Moreover, overexpression of TERT could reverse AO40-induced telomerase activity inhibition, β-galactosidase activity, and the decrease of cell viability.

We also evaluated the effect of AO42 and SAO42 on HeLa cells. Compared to SAO42, the AO40 and AO42 inhibited telomerase activity more effectively, though these three oligomers had comparable cytotoxicity on HeLa cells (Figure S11). These results were similar to those on PC12 cells, further supporting β-sheet-rich Aβ oligomers could be potent telomerase inhibitors.

To provide more direct evidence for the telomerase inhibition by Aβ in cells, we constructed a eukaryotic plasmid for expression of Aβ1-42. As shown in Figure 6a, the telomerase activity in PC12 cells transfected with Aβ1-42 was obviously decreased, indicating that Aβ1-42 could inhibit telomerase activity intracellular. We next investigated the intracellular localization of Aβ1-42 by using immunofluorescence assay. After 48h transfection, the Aβ1-42 entered into the nucleus and especially localized at the telomere, as clarified by the colocalizations with telomere binding protein TRF1, an effective marker for interphase telomeres (Figure 6b).[67,68]

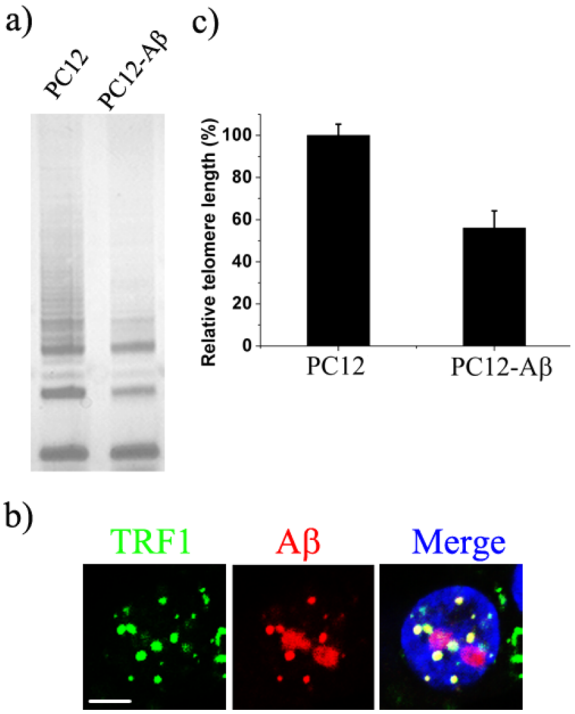

Figure 6. Intracellular Aβ located at telomere and inhibited telomerase activity in cellular environments. (a) The intracellular Aβ induced telomerase activity inhibition. (b) Immunofluorescence experiment for TRF1 (green) and Aβ42 (red) in PC12-Aβ cells. Scale bar = 5 μm. (c) The telomere lengths of PC12 cells and stable transfected PC12-Aβ cells detected by qRT-PCR.

These results supported the conclusion that Aβ inhibited telomerase activity intracellular.

To further study Aβ inhibition effect on telomerase, we generated a stable transfected PC12 cell line which could stably express Aβ1-42. After 30 days of neomycin selection, the telomere length of transfected PC12-Aβ cells and PC12 cells was measured by using quantitative real-time polymerase chain reaction (qRT-PCR). The telomere length of PC12-Aβ cells decreased 45% compared with PC12 cells (Figure 6c). In addition, the transfected PC12-Aβ cells showed morphological abnormity and slow cell proliferation, suggesting the decreased tumorigenic ability caused by telomerase activity inhibition (Figure S12).

DISCUSSION

Until now, the exact pathological mechanisms of Aβ are not clear. More and more studies have demonstrated that soluble oligomers or protofibrils of Aβ are active species that ultimately cause the synaptic loss and dementia associated with AD.[11,69−71] Here we found that the aggregated Aβ (AO40 and AO42) could effectively inhibit telomerase activity in vitro and intracellularly. Several lines of evidence demonstrate the β-sheet structures play an important role in telomerase inhibition: (1) the aggregated Aβ with β-sheet structures could inhibit telomerase activity, but the Aβ monomers could not; (2) curcumin, which could disrupt the β-sheet structures formation, could prevent the Aβ-induced telomerase activity inhibition; and (3) the SAO42, which had similar size and morphologies with AO42, but a lack of β-sheet structures, could not inhibit telomerase activity. The structure conversion of Aβ is complex and may take different multiple pathways associated with concentration, buffer condition, and temperature, etc. The polymorphism of Aβ aggregation and related molecular mechanism of their cytotoxicity are not clear at present.

It was reported that the β-sheet structures of Aβ correlated with neurotoxic activity.[13] We observed the correlation between the telomerase inhibition capacity and the neurotoxicity for β-sheet-rich Aβ oligomers, suggesting the telomerase inhibition was involved in Aβ-induced cytotoxicity. However, we did not observe this correlation with the SAO42 sample. Previous studies have indicated that different conformations of Aβ species caused neurotoxicity by distinct mechanisms.[43] It is well-known that cell death in AD is caused by combination of many complex biological events.[3] Studies have demonstrated a series of molecular mechanisms of Aβ oligomers toxicity, including receptor-mediated neurotoxicity, cellular membrane damage, oxidative stress, and elevations in Ca^{2+} et al.[71] Telomerase inhibition may also play an important role in Aβ-induced cytotoxicity for several reasons: (1) the observed correlation between telomerase inhibition and cytotoxicity for AO40, AO42, and other fragments of Aβ peptides; (2) Aβ oligomers could act as potent telomerase inhibitor and induce cellular senescence and decrease of cell viability on HeLa cells; (3) recovery of the telomerase activity could reverse the cytotoxicity induced by Aβ oligomers; and (4) the intracellular Aβ could localize at the telomere and induce telomerase inhibition and shortening. All these results indicate that telomerase inhibition may be involved in the pathological mechanism of AD and correlate with Aβ-induced cytotoxicity.

The interaction between Aβ and DNA/RNA has long been known. Our group found that Aβ could induce the DNA conformation transition.[48,49] Recently, sequence-specific interaction between intracellular Aβ and DNA has been reported.

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Ohyagi et al. indicate that Aβ bound to "GGATTGGGGT" oligonucleotides in a p53 promoter in a sequence-specific manner.[52] Maloney et al. further identified an Aβ-interacting domain "KGGRKTGGGG".[54,41] The sequence of RDH was "GTTAGGGTTAG", also a G-rich sequence. Our results suggested that the specific Aβ-RDH interactions played an important role in the Aβ related physiological and pathological mechanisms. In addition, the consecutive "G" sequence may be the key factor to influence the Aβ-DNA/RNA interaction.

It should be pointed out that the precise mechanism for Aβ-induced telomerase activity inhibition and the consequent events are still not completely understood. Under which physiological conditions does Aβ control telomerase? Recently, a growing body of evidence suggests that accumulation of intraneuronal oligomeric Aβ is an early event in the pathogenesis of AD.[72−75] Heat and oxidative stress may induce the intracellular location of Aβ peptides.[76,77] The nuclear localization of Aβ aggregates has also been reported in CHO cells and AD brain samples.[21,52,78−80] Our results showed that the Aβ was not only found in nuclei but also located at telomeres.

In summary, we demonstrated that aggregated Aβ could directly inhibit human telomerase activity in vitro and living cells. The β-sheet structure of Aβ oligomers played an important role in the Aβ-induced telomerase activity inhibition. Aβ oligomers blocked telomerase reaction through binding to the telomeric synthetic template RDH. Further study showed that intracellular Aβ could locate at a telomere and induce a rapid telomerase activity inhibition and telomere loss. Our work provides new insights into Aβ-induced neurotoxicity. It is well-known that aging is the highest risk factor for AD. In view of the central role of telomere and telomerase in the aging process, our study may offer a new clue to better understand aging and AD.

MATERIALS AND METHODS

Aβ Sample Preparation. Aβ1-40 and Aβ1-42 were purchased from American Peptide Company. Peptide fragments of Aβ including Aβ1-12, Aβ12-28, and Aβ25-35 were purchased from Sigma Chemical. Methods for preparing aggregated Aβ peptides were as previously described.[81] Briefly, the powered Aβ peptide was first dissolved in 1,1,1,3,3,3-hexa fluoro-2-propanol (HFIP) at the concentration of 1 mg/mL. The solution was shaken at 4 °C for 2 h in a sealed vial for further dissolution and was then stored at −20 °C as a stock solution. Before use, the solvent HFIP was removed by evaporation under a gentle stream of nitrogen, and peptide was dissolved in water. Fresh dissolved Aβ1-40 was defined as monomer. Aβ1-40 oligomers, protofibrils, and fibrils were prepared at 37 °C for 3, 5, and 8 days, respectively, in 20 mM Tris, pH 7.4 buffer, at 80 μM.

Aβ1-42 was dissolved in NaOH (0.5 mM), sonicated (30s) and diluted in phosphate-buffered saline (PBS) (50 mM NaH2PO4, 100 mM NaCl, pH 7.4). The peptide was then centrifuged (13,000 rpm for 20 min), and the supernatant was used in the following experiment. Aβ1-42 oligomers (AO42) were prepared in PBS at 37 °C for 6 h at 100 μM. Stable Aβ1-42 oligomers (SAO42) were prepared in PBS at 25 °C for 1 day at 25 μM.

Peptide fragments of Aβ including Aβ1-12, Aβ12-28, and Aβ25-35 were incubated under different conditions per manufacturer's recommendations, indicating the oligomers formation under these conditions.

In Vitro Telomerase Activity Assay. Telomerase extract from PC12 cells (rat pheochromocytoma, American Type Culture Collection) was prepared as described previously.[82] For TRAP assay, the telomerase extracts (3 μg) were added to 40 μL of RNA secure pretreated extension solution containing 1 × TRAP buffer, (20 mM Tris-HCl, pH 8.3, 1.5 mM MgCl2, 63 mM KCl, 0.005% Tween 20, 1 mM EGTA, BSA 0.1 mg/mL) 1 mM dATP, dTTP, dCTP, and dGTP, and 0.1 μg TS primer (AATCCGTCGAGCAGAGTT) with different doses of various Aβ peptides at 30 °C for 60 min and then 90 °C for 5 min to stop the reaction. CX primer (0.1 μg CCCTTACCCT-TACCCTTACCCTAA), 0.1 μg TSNT primer (AATCCGTCGAG-CAGAGTTAAAAGGCCGAGAAGC), and 0.1 μg NT primer (ATCGCTTCTCGGCCTTTT) were added further for polymerase chain reaction (PCR) (30 cycles; 94 °C, 30 s; 60 °C, 30 s; 72 °C, 60 s). Finally, 15 μL of the PCR product was loaded to 12% nondenatured PAGE and visualized by silver staining.

ASSOCIATED CONTENT

Supporting Information

Materials and Methods in detail. Curcumin prevents the Aβ-induced telomerase inhibition. AO40 inhibit telomerase activity dose-dependent. Oligomers of various peptide fragments inhibit telomerase activity. Recovery experiment to determine that the Aβ inhibit telomerase activity through binding to RDH. Aβ oligomers destabilized the RDH. The binding mode of Aβ bound to RDH studied by competitive binding assay. The effect of various peptide fragments of Aβ on cell viability and telomerase activity inhibition. Morphological abnormity and slow cell proliferation of transfected PC12-Aβ cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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