Synthesized Aβ42 Caused Intracellular Oxidative Damage, Leading to Cell Death, via Lysosome Rupture

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ABSTRACT. Neuronal cellular accumulation of amyloid beta peptide (Aβ) has been implicated in the pathogenesis of Alzheimer’s disease (AD). Intracellular accumulation of Aβ42, a toxic form of Aβ, was observed as an early event in AD patients. However, its contribution and the cellular mechanism of cell death remained unclear. We herein revealed the mechanism by which Aβ42 incorporated into cells leads to cell death by using chemically synthesized Aβ42 variants. The Aβ42 variant Aβ42 (E22P) which has an increased tendency to oligomerize, accumulated in lysosomes at an earlier stage than wild-type Aβ42, leading to higher ROS production and lysosomal membrane oxidation, and resulting in cell death. On the other hand, Aβ42 (E22V), which is incapable of oligomerization, did not accumulate in cells or affect the cell viability. Moreover, intracellular localization of EGFP-Galectin-3, a β-galactoside binding lectin, showed that accumulation of oligomerized Aβ42 in lysosomes caused lysosomal membrane permeabilization (LMP). Overexpression of lysosome-localized LAMP1-fused peroxiredoxin 1 and treatment with U18866A, an inhibitor of cholesterol export from lysosomes that causes an increase in lysosomal membrane stability, attenuated Aβ42-mediated LMP and cell death. Our findings show that lysosomal ROS generation by toxic conformer of Aβ led to cell death via LMP, and suggest that these events are potential targets for AD prevention.

Key words: Amyloid-beta (Aβ), Cell death, Lysosome, Lysosomal membrane permeabilization, Reactive oxygen species (ROS)

Introduction

Amyloid beta peptide (Aβ) has been observed as the main component of deposits in the brains of Alzheimer’s disease (AD) patients (Selkoe, 2008). Cellular accumulation of Aβ has been associated with the pathogenesis of AD (Selkoe, 2008). Aβ, which is typically composed of 39–43 amino acids, is produced by cleavage of amyloid precursor protein (APP) by three (α-, β- and γ-) secretases in the extracellular space. Major species of Aβ are Aβ40 and Aβ42 (Selkoe, 2000). Aβ42 has a higher tendency to oligomerize and is more toxic to neuronal cells than Aβ40. The increased ratio of Aβ42/Aβ40 during aging has been implicated in the pathogenesis of AD (Benilova et al., 2012).

The tendency of Aβ42 to oligomerize together with the generation of reactive oxygen species (ROS) by Aβ42 was associated with oxidative stress and cell death (Butterfield and Boyd-Kimball, 2005; Murakami et al., 2011). Elevated levels of ROS have been found in early onset of AD brains (Zhu et al., 2004; Pratico, 2002). Aβ42 has also been shown to generate radicals in vitro through electron transfer between Tyr10 and Met35 during the process of oligomerization (Murakami et al., 2005). The rate of ROS production is dependent on the oligomerization tendency of Aβ42 species (Murakami et al., 2005). Substitutions of specific amino acids in Aβ42 affect this property. An E22P substitution stabilized a turn structure formed between two β-sheet structures in Aβ42, and facilitated the formation of oligomers, resulting in higher toxicity than wild-type Aβ42 (Murakami et al., 2003; Masuda et al., 2009). In contrast,
Materials and Methods

Intraneuronal accumulation of Aβ has been observed as an early event in AD patients and has been implicated in AD pathology (Gouras et al., 2010; Liu et al., 2010). Aβ42 produced innately in the extracellular space is incorporated into cells through endocytosis using endocytosis receptors such as low-density lipoprotein receptor-related protein 1 (LRP1) (Fuentelahla et al., 2010; Nagle et al., 2002; Snyder et al., 2005). Intraneuronal Aβ42 has been shown to accumulate in the endosome/lysosomes (Fuentelahla et al., 2010), and accumulated Aβ42 induced lysosomal membrane permeabilization (LMP) leading to apoptosis (Ditaranto et al., 2001; Liu et al., 2010). In APP transgenic mice or APP-transfected cells, APP and Aβ42 accumulated in mitochondria, causing mitochondrial dysfunction including inhibition of electron transfer chain complexes, which in turn caused ROS generation and resulted in neurotoxicity (Leuner et al., 2012; Du et al., 2010; Langui et al., 2004). However, overexpression of APP might cause mislocalization of APP, and as such, the toxic effects of Aβ derived from overexpressed APP on cellular homeostasis are difficult to evaluate. In addition, Aβ42 has also been observed in autophagosomes in neurons of AD patients (Nixon et al., 2000, 2005), which has also been implicated in Aβ42 metabolism (Lunemann et al., 2007; Güglielmotto et al., 2014; Nilsson et al., 2013).

Although many reports have documented the intracellular accumulation of Aβ42 and its involvement in AD pathogenesis, the precise cellular mechanism by which intracellular accumulated Aβ42 causes cell death remains unclear. Here, we investigated the intracellular accumulation of chemically synthesized Aβ42 in SH-SY5Y neuroblastoma cells using Aβ42 derivatives (wild type, E22P and E22V). Our results revealed that synthesized A β42 accumulated within lysosomes and that ROS generated caused LMP, resulting in cell death.

Cell Culture, Construction of Plasmids, and Transfection

Human SH-SY5Y neuroblastoma cells were obtained from ATCC and cultured in Dulbecco’s Modified Eagle’s Medium (GIBCO) supplemented with 10% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). Cells were incubated under humidified air with 5% CO₂ at 37°C. Ptx1 and LAMP1-Prx1 expression plasmids were constructed as follows: Total RNA was extracted from cells with an RNeasy Mini Kit (Qiagen) and reverse-transcribed with ReverTraAce enzyme (TOYOBO). The resultant cDNA was used as a template for the amplification of prx1 (forward: 5'-ggatcatgtagttggaatgtaatgccac-3', reverse: 5'-ggaatctcacttctgctt-ggagaaatattctttgct-3') and LAMP1 (forward: 5'-ggagcctcagctgggaaaccgggagc-3', reverse: 5'-ggttctgtagtgctgatcgtgagcactggt-3'). To construct an expression plasmid expressing LAMP1-prx1, the amplified prx1 was subcloned into the BamHI-EcoRI sites of pcDNA3.1 (+) (Invitrogen) and then the amplified LAMP1 was subcloned into the KpnI-BamHI sites of pcDNA3.1 (+) containing the prx1 gene. The EGFP-Gal3 expressing plasmid (pEGFP-hGal3, (Maejima et al., 2013)) was kindly provided by Tamotsu Yoshimori (Osaka University). Site-directed mutagenesis of Ptx1 was performed using PrimeSTAR Max DNA polymerase (Takara) and the following primers: forward: 5'-ttgctgaggcagctggatcgttgc-3', reverse: 5'-ctgctgctggaag-3'. Cells were transfected with plasmids using Lipofectamine 2000 (Invitrogen) unless otherwise noted.

Reagents and Antibodies

U18866A was purchased from Sigma. Mouse monoclonal anti-Aβ antibody 11A1 (specific to toxic conformer of Aβ) was prepared as described previously (Murakami et al., 2010). Rabbit monoclonal antibodies against Cathepsin B (EPR4323) and anti-LAMP1 (EPR4204) from Abcam and against rab5A (EPR5438) and LC3B (D11) from Cell Signaling were used. Mouse monoclonal antibodies against Ptx1 (LS-C61912), β-actin (A5441) and GFP (JL-8) were purchased from LSBio, Sigma-Aldrich and Clontech, respectively.

Synthesis and Preparation of Aβ Peptide and Incubation of Aβ Peptide with Cells

Aβ42 derivatives were synthesized as described previously (Murakami et al., 2002). Synthesized Aβ42 peptides were incubated for 30 min on ice in cold 1,1,3,3,3-hexafluoro-2-propanol (HFIP) and then HFIP was evaporated by vacuum centrifugation, and samples were stored at –80°C before use. Aβ peptides added in the medium were incubated with cells all through indicated times.

Fluorescence Microscopy

All fluorescence images were obtained using a LSM510 META confocal microscope (Carl Zeiss). To detect lysosomal ROS, cells were incubated with 2 μM RedoxSensor™ Red CC-1 and 50 nM LysoSensor Green™ DND-187 as a lysosomal marker (both Invitrogen) at 37°C for 30 min in the dark. For observation of lysosomal membrane peroxidation, cells were incubated with 100 nM BODIPY® 581/591 C11 (Lipid peroxidation sensor) and 50 nM LysoTracker DeepRed as a lysosomal marker (both Invitrogen) for 45 min in the dark. For immunostaining, cells were fixed in 4% paraformaldehyde at room temperature (r.t.) for 20 min, and then incubated for blocking and permeabilization in blocking buffer (1% glyceral, 1% bovine serum albumin, 1% normal goat serum, and 0.2% Triton X-100 in PBS (−)), phosphate buffered saline without calcium and magnesium) for 1 h. The fixed and per-
meabilized cells were incubated with primary antibodies overnight at 4°C, and then with Alexa Fluor 488- or 543-conjugated goat anti-mouse or rabbit IgG as secondary antibodies for 1 h.

**Quantification of colocalization analysis of EGFP-Gal3 with LAMP1**

To increase transfection efficiency in this experiment, transfection to SH-SY5Y cells was performed using the Amaxa Nucleofector system according to amaxa™ 4D-Nucleofector Protocol for SH-SY5Y. At 48 h post-transfection with the EGFP-Gal3 plasmid, NAC or U18666A was pretreated for 24 h and then the cells were incubated with 2 μM of Aβ42 for 12 h. For double transfection of EGFP-Gal3 and LAMP-1, cells were first transfected with EGFP-Gal3 plasmid. At 48 h post-transfection, the plasmid expressing LAMP1-Prx1 was transfected. After 24 h of the second transfection, the cells were incubated with 2 μM of Aβ42 for 12 h. Cells were fixed and immunostained cells and then fifty cells were analyzed for each group and the cells showing EGFP-Gal3 foci merged with LAMP1 (positive dots) were counted.

**MTT Assay**

Cell viability was determined by the MTT assay as previously described (Maharjan et al., 2014). Cells grown in a 96-well microplate were incubated with 0.25 mg/ml MTT (Nacalai Tesque) for 4 h at 37°C and then precipitated formazan was dissolved in isopropanol with 0.04 mol/l HCl for 30 min at r.t. The absorbance at 570 nm was measured by using a Sunrise™ absorbance plate reader (TECAN).

**Results and Discussion**

**Aβ42 incorporated in cells accumulated as toxic conformer including oligomer in lysosomes**

To investigate the intracellular localization of Aβ42 incorporated from the extracellular space, SH-SY5Y cells were incubated with chemically synthesized Aβ42 peptides and their localization was analyzed by immunofluorescence using confocal microscopy (Fig. 1). Intracellular wild-type Aβ42 was immunostained with Aβ42 antibody (11A1), which specifically recognizes a turn structure specific to toxic conformer of Aβ42 (Murakami et al., 2010). The wild-type Aβ42 appeared as distinct foci after 12- and 16-h incubation (Fig. 1A). The foci co-localized with the lysosomal marker protein LAMP1. The foci of the Aβ42 (E22P) mutant, which has a higher oligomerization tendency than the wild type (Masuda et al., 2009; Izuo et al., 2012), were detected after 8-h of incubation, which was earlier than the wild-type Aβ42, and these foci also merged with LAMP1 (Fig. 1B). In addition, large foci co-localized with LAMP1 seem to localize to perinuclear regions under incubation with Aβ42, although smaller foci did not (Supplementary Fig. 1). In contrast, the intracellular immunofluorescence signal of the Aβ42 (E22V) mutant, which has a weaker oligomerization tendency compared to the wild-type (Murakami et al., 2005), was not detected even after 24-h of incubation (Fig. 1C). These results suggest that Aβ42 incorporated in cells was accumulated as toxic conformer including oligomers in lysosomes, which correlated with the oligomerization property of Aβ42.

**Cytotoxicity of Aβ42 correlated with its lysosomal accumulation**

Next, the effect of intracellular Aβ42 accumulation on cell viability was assessed by using the MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) assay (Fig. 2). SH-SY5Y cells were incubated with wild-type Aβ42, Aβ42 (E22P) or Aβ42 (E22V) for the indicated time periods. Treatment with wild-type Aβ42 decreased cell viability in a time-dependent manner and viability decreased by about 25% at 16 h. Aβ42 (E22P), which accumulated in lysosomes earlier than the wild-type Aβ42 (Fig. 1), decreased cell viability by 20% at 8 h, by 30% at 12 h, and by 40% at 16 h, earlier than the wild type, in a time-dependent manner. The effect of Aβ42 (E22P) on cell viability was more severe than that of wild-type Aβ42. In contrast, Aβ42 (E22V), which was not detected as toxic conformer in lysosomes (Fig. 1C), did not affect cell viability even after a 16-h incubation. These results suggest that the cytotoxicity of Aβ42 to SH-SY5Y cells is associated with its accumulation in lysosomes, which parallels its oligomerization tendency.

**Lysosomal accumulation of toxic conformer Aβ42 caused ROS generation and membrane lipid oxidation**

To reveal the mechanism underlying cell death due to lysosomal accumulation of Aβ42, ROS generation in lysosomes was monitored using a redox indicator, RedoxSensor CC-1 (2,3,4,5,6-pentafluoro-dihydrotetramethylrosamine, PF-TMRos) (Chen and Gee, 2000) (Fig. 3A). RedoxSensor CC-1 generates red-fluorescence upon oxidation by ROS and also changes intracellular localization in a cytosolic redox state-dependent manner: When the cytosolic redox state is normal (reducing), RedoxSensor CC-1 localizes in lysosomes. In contrast, when the cytosolic redox state becomes oxidized, RedoxSensor CC-1 is localized in mitochondria (Chen and Gee, 2000). The pH indicator LysoSensor Green was used as a lysosome marker, because it exhibits fluorescence only in an acidic compartment. Incubation with Aβ42 (E22P) caused significant red fluorescence of RedoxSensor CC-1 after 8-h, when lysosomal accumulation of Aβ42 (E22P) was observed. And at 8 h, most of the red fluorescence of Redox Sensor CC-1 co-localized with green fluorescence from LysoSensor Green,
Incubation with wild-type Aβ42 resulted in weak fluorescence of RedoxSensor CC-1, which did not merge well with the fluorescence of LysoSensor Green at 8 h (Fig. 3A, left). Further incubation with wild-type Aβ42 resulted in foci of RedoxSensor CC-1 after 12 and 16 h, which mostly merged with the fluorescence of LysoSensor Green. At these time points, lysosomal accumulation of wild-type Aβ42 was observed (Fig. 3A, left and Fig. 1A). At 16 h incubation with the wild-type Aβ42 and Aβ42 (E22P), some RedoxSensor CC-1 foci did not merge with LysoSensor Green (see magnified figures in Fig. 3A). In contrast, Aβ42 (E22V), lysosomal accumula-
tion of which was not detected (Fig. 1C), caused only faint fluorescence of RedoxSensor CC-1 at all examined incubation times (Fig. 3A, right). These data suggest that lysosomal accumulation of toxic conformer Aβ42 caused ROS generation in lysosomes.

To observe lysosomal membrane lipid oxidation caused by Aβ42-mediated ROS, we used BODIPY 581/591 C11, which is widely used for detection of membrane lipid oxidation based on the shift of its fluorescence emission from red (Ex 543) to green (Ex 488) (Pap et al., 1999). Incubation with wild-type Aβ42 for 12 h substantially increased the green fluorescence of BODIPY 581/591 C11, which merged with the lysosome marker LysoTracker DeepRed, indicating oxidation of lysosomal membranes (Fig. 3B and Supplementary Fig. 2). Aβ42 (E22P) significantly increased the green fluorescence ratio (Ex 543/Ex 488) of BODIPY 581/591 C11, which merged with the lysosome marker LysoTracker DeepRed. Quantification of the relative fluorescence ratio (Ex 543/Ex 488) of BODIPY 581/591 C11 showed that wild-type Aβ42 and Aβ42 (E22P) caused oxidation of lysosomal membrane lipids and the extent of oxidation was more significant for Aβ42 (E22P) compared with wild-type Aβ42 (Fig. 3C). These results suggest that Aβ42 accumulated in lysosomes yielded ROS and caused membrane lipid oxidation in lysosomes, depending on the oligomerization tendency of the Aβ42 species.

Antioxidant treatment and lysosomal expression of an antioxidative enzyme suppressed Aβ42-mediated cell death

To clarify the relationship of lysosomal ROS generation by oligomerized Aβ42 with cell death, we first examined the effect of N-acetylcysteine (NAC) on Aβ42-mediated cell death. NAC is a precursor of glutathione and pretreatment of cells with it increases intracellular glutathione levels. Cells were pretreated with 1 mM NAC for 24 h, and then incubated with Aβ42 and Aβ42 (E22P) for 12 h. Pretreatment with NAC slightly improved cell viability after incubation with Aβ42 (Fig. 3D). Next, we investigated the effect of LAMP1-Preoxiredoxin (Prx1), a thioredoxin-dependent peroxidase targeted to the cytosolic side of the lysosomal surface. Overexpression of LAMP1-Prx1 significantly increased the cell viability upon incubation with wild-type Aβ42 as well as after that with Aβ42 (E22P) (Fig. 3E). In contrast, overexpression of cytosolic Prx1 or LAMP1-Prx1 C52A lacking peroxidase activity did not restore cell viability, although the expression levels of LAMP1-Prx1 and its C52A mutant were comparable and the immunofluorescence of LAMP1-Prx1 merged with that of the lysosome marker cathepsin B (Supplementary Fig. 3). These results indicate that overexpression of an antioxidative enzyme at the lysosomal surface suppressed Aβ42-mediated cell death.

Depending on the oligomerization tendency of the Aβ42 variants, lysosomal ROS generation and membrane oxidation were increased, and then cell death was induced. Aβ42 generates radicals in vitro in an oligomerization-dependent manner (Murakami et al., 2005). Therefore, toxic conformer Aβ42 including oligomer accumulated in lysosomes appears to be directly responsible for ROS generation, leading to lysosomal membrane oxidation, followed by cell death.

Toxic conformer of Aβ42 in lysosomes induced lysosomal membrane permeabilization

Intracellular ROS generation could cause lysosomal membrane permeabilization (LMP) leading to cell death (Boya and Kroemer, 2008). We investigated whether accumulation of toxic conformer of Aβ42 in lysosomes induces LMP by detecting the localization of EGFP-Galectin-3 (Gal3), which is widely used as a marker of ruptured secretory vesicles or organelles (Maejima et al., 2013). Gal3 recognizes the β-galactoside residues that are present on the luminal side of post-Golgi compartment membranes. Therefore, EGFP-Gal3 could be localized inside of damaged post-Golgi compartments. On the other hand, EGFP-Gal3 is diffuse in the cytosol under normal conditions as shown in Fig. 4A. Incubation with Aβ42 or Aβ42 (E22P) for 12 h resulted in foci of EGFP-Gal3 that merged with LAMP1 (Fig. 4A). Foci of EGFP-Gal3 were partially merged with an autophagy marker LC-3B (Supplementary Fig. 4). Large aggregated lysosomes seemed to localize to perinuclear regions (Fig. 1A and 1B). Perinuclear clustering of lysosomes under starvation promoted autophagosome formation and autophagosome-fusion rate (Korolchuk et al., 2011). Therefore, perinuclear localization of lysosomes following lysosomal accumulation of Aβ42 might promote...
Fig. 3. Toxic conformer of Aβ42 caused lysosomal oxidation and cell death. (A) After incubation with 2 μM Aβ42 derivatives for the indicated times, cells were stained with RedoxSensor™ Red CC-1 to detect intracellular ROS and LysoSensor Green as a lysosome marker. Magnified images were shown in lower panel. (B) To measure lysosomal membrane oxidation, after incubation with 2 μM Aβ42 derivatives for 12 h cells were incubated with BODIPY 581/591 C11 and LysoTracker DeepRed as a lysosome marker. Oxidized BODIPY was excited at 488 nm and detected with a band-path filter (505–530 nm) and reduced BODIPY was excited at 543 nm and detected with a band-path filter (560–615 nm). LysoTracker DeepRed was excited at 633 nm and detected with a band-path filter (636–722 nm). Square portions were magnified in Supplementary Fig. 2. (C) Relative fluorescence intensity ratio of BODIPY 581/591 C11 (Oxi/Red). Quantified ratios are shown as the means±s.e.m. of three independent measurements. *P<0.05 and **P<0.01 vs. control. (D) Pretreatment of NAC slightly decreased Aβ42-mediated cell death. PBS (phosphate buffered saline) was used as solvent for NAC. Data are represented as relative values with respect to a PBS control. Quantified values are shown as the means±s.e.m. of three independent experiments. *P<0.05 vs. control in each condition. (E) Lysosomal localized antioxidative protein Prx1 suppressed Aβ42-mediated cell death. Quantified ratios are shown as the means±s.e.m. of three independent measurements. *P<0.05 and **P<0.01 vs. control.
Fig. 4. Toxic conformer of Aβ42 caused lysosome membrane permeabilization. Immunofluorescence analysis of cells expressing EGFP-Gal3 after incubation of Aβ42 derivatives at 12 h. EGFP-Gal3 co-localized with the lysosome marker LAMP1 (A). Effect of antioxidation (overexpression of LAMP1-Prx1 (B) and NAC treatment (C)) on EGFP-Gal3 foci formation under 12 h incubation with the wild-type Aβ42. Fluorescence images were shown in upper panel and EGFP-Gal3 positive dots (EGFP-Gal3 foci merged with LAMP1) per cells were quantified in lower panel. (D) U18666A (UA) treatment prevented Aβ42-mediated cell death. Cells were pretreated with 3 μM UA for 24 h to inhibit cholesterol transport from lysosomes to the Golgi and ER, and then incubated with Aβ42 and Aβ42(E22P) for 12 h. (F) U18666A treatment prevented Aβ42-mediated EGFP-Gal3 foci formation (fluorescence image (left) and quantification of EGFP-Gal3 positive dots per cell (right)). Quantified values are shown as the means±s.e.m. of three independent experiments. *P<0.05 vs. mock control in (B). **P<0.01 vs. DMSO (control) for each condition in (D).
lysophagy. These results suggest that Aβ42 accumulation induced membrane rupture in lysosomes and partial rupture in endosomes. Intracellular accumulation of Aβ42 in APP transgenic mice was reported to cause lysosomal and endosomal leakage (Almeida et al., 2006; Umeda et al., 2011). LMP causes the release of various lysosomal hydrolytic enzymes such as cathepsin from the lysosome to the cytosol, and the leaked cathepsin activates various types of cell death signaling, including ROS generation (Erdal et al., 2005; Boya and Kroemer, 2008). Lysosomal accumulation of α-synuclein also led to LMP and endosome membrane rupture, leading to an increase in cathepsin B-dependent intracellular ROS and cell death (Freeman et al., 2013). Notably, Aβ42-mediated cell death could be attenuated by LAMP1-Prx1 in an enzyme-activity-dependent manner, but not by diffuse cytosolic Prx1. Furthermore, overexpression of LAMP1-Prx1 significantly decreased EGFP-Gal3 foci merged with LAMP1 (Fig. 4B) and decrease of EGFP-Gal3 foci by NAC treatment was close to significance (p=0.058) (Fig. 4C), although aggregated lysosomes were still observed in the cells incubated with Aβ42. Therefore, Aβ42-mediated ROS generation in lysosomes is suggested to promote LMP and cell death.

To clarify the relationship between LMP and cell death after Aβ42-treatment, we investigated the effect of U18666A, which has been reported to attenuate LMP (Reiners et al., 2011), on Aβ42-mediated cell death. U18666A inhibits cholesterol transport from the lysosomal membrane to the Golgi or endoplasmic reticulum, resulting in a decrease in lysosomal membrane fluidity and an increase in lysosomal membrane stability by increasing the cholesterol content in the lysosomal membrane (Appelqvist et al., 2011). U18666A pretreatment significantly restored cell viability after incubation with wild-type Aβ42 or Aβ42 (E22P) (Fig. 4D). Furthermore, U18666A pretreatment significantly decreased EGFP-gaI3 foci merged with LAMP1 (Fig. 4E). These results suggest that Aβ42 caused LMP, leading to cell death.

In conclusion, with the use of chemically synthesized Aβ42, we demonstrated that intracellularly incorporated Aβ42 accumulated in lysosomes as toxic conformer including oligomer. The oligomerization tendency of Aβ42 enhanced the accumulation of Aβ42 in lysosomes, which resulted in ROS generation in lysosomes, LMP, and finally cell death (Fig. 5). Prx1 anchored to lysosomes could eliminate ROS resulting from Aβ42 in lysosomes, and U18666A pretreatment could suppress cell death caused by Aβ42-accumulation. Therefore, elimination of ROS caused by Aβ42 in lysosomes and inhibition of LMP might be potential measures to prevent the onset of AD.

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Conflicts of interest

The authors declare that there is no conflicts of interest.

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