Mitochondrial perturbation drives tau oligomers pathology in Alzheimer's disease

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Article

Keywords: tau oligomers, mitochondrial reactive oxygen species, Alzheimer's disease

DOI: https://doi.org/10.21203/rs.3.rs-40746/v1

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Abstract

Tau oligomers, prior to neurofibrillary tangle formation, are toxic species responsible for tau pathology, mitochondrial and synaptic damage, and memory impairment. The underlying mechanisms of abnormal tau accumulation and strategies to eliminate them remain largely unknown. The present study addresses whether mitochondrial reactive oxygen species (ROS) are major contributing factors for tau oligomer formation and, if so, whether eliminating mitochondrial ROS reduces accumulation of tau oligomers and improves mitochondrial and cognitive function in Alzheimer’s disease (AD). First, we determined whether increased oxidative stress correlates with aggregation of tau oligomers in human AD-affected brains, Aβ/tau overexpressed mouse models, human trans-mitochondrial “cybrid” (cytoplasmic hybrid) neuronal cells containing mild cognitive impairment (MCI) and AD-derived mitochondria, and Aβ/tau expressing neuronal cells. In P301S tau and AD mice, upregulation of tau oligomers correlates with ROS accumulation. Elevated tau oligomer levels are also correlated with elevated ROS levels in the AD patient hippocampus. Importantly, human cybrid cells, whose mitochondria are derived from platelets of patients with sporadic AD or MCI, displayed aggregated tau oligomers, which also correlated with upregulated ROS levels. Application of mito-Tempo, a mitochondria-targeted antioxidant, to inhibit the generation of mitochondrial and intracellular ROS in tau and AD neurons, as well as in MCI and AD cybrids ex vivo, leads to a striking decrease in tau oligomers. Finally, in AD mice, mito-Tempo inhibited tau oligomer accumulation and improved behavioral deficiency. Our work adds to the growing body of evidence that oxidative stress contributes to tau oligomer formation and that inhibition of oxidative stress ameliorates tauopathy in AD.

Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disease that is associated with abnormal upregulation of oxidative stress. A key pathological hallmark of AD is the presence of neurofibrillary tangles (NFTs), which are composed of hyperphosphorylated aggregates of tau. Elevated phosphorylation and aggregation of tau destabilizes tau-microtubule interactions, leading to microtubule instability, dysfunctional axonal transport along microtubules, and neuronal death. Tau phosphorylation is modulated by stress conditions such as oxidative stress and alterations in glucose metabolism during hypothermia and starvation. Tau oligomers, intermediate species that form prior to NFTs, include various species of tau protein including dimeric, multimeric, granular and possibly small filamentous aggregates, have deleterious effects on synaptic function and contribute to memory deficiency.

Mitochondria are a major source of reactive oxygen species (ROS). Oxidative stress is a pathological characteristic of tauopathy and evidence has shown that accumulation of ROS could directly stimulate tau hyperphosphorylation and aggregation. However, the precise role of tau oligomers in the disease process is poorly understood. In the present study, we investigated accumulation of pathological tau oligomers and its relevance to AD pathogenesis including tau and amyloid pathology and dysfunctional
AD mitochondria to address the following key questions: does the accumulation of tau oligomers associate with mitochondrial defects and ROS production in AD and an Aβ/tau-enriched environment? If so, does blockade of mitochondrial ROS eliminate tau oligomer formation and attenuate mitochondrial dysfunction? Does suppression of mitochondrial ROS rescue Aβ-mediated cognitive dysfunction? We comprehensively analyzed levels of tau oligomers, ROS, mitochondrial function, and behavioral endpoints using AD patient hippocampus, tauopathy and human Aβ-producing AD mouse models and in vitro cultured neurons, and MCI and AD cybrids as ex vivo models for AD mitochondrial dysfunction. Our studies demonstrate that tau oligomers were significantly elevated in the AD patient hippocampus, P301S tauopathy and mAPP mice hippocampus and entorhinal cortex, cultured neurons, and human MCI and AD cybrids. Importantly, there is a positive correlation between tau oligomer levels and ROS levels. Scavenging mitochondrial ROS prevented accumulation of toxic tau oligomers, attenuated Aβ- and tau-induced mitochondrial perturbation, and led to improvements in learning and memory in P301S tauopathy and mAPP mice.

**Results**

**Accumulation of tau oligomers is associated with ROS in the AD brain**

Given the accumulation of amyloid beta (Aβ) and abnormal tau in AD-affected brain regions including the hippocampus, we first tau oligomers (oTau) in the AD-affected hippocampus. Immunostaining with specific tau oligomeric complex I (TOC1) antibody showed that intensities of oTau-positive signals were significantly elevated by 3–4 folds in the AD hippocampus (Fig. 1A-B). Consistent with the immunostaining results, immunodot blotting demonstrated that oTau levels were greatly elevated in the AD hippocampus but not in the cerebellum as compared with non-AD brains (Fig. 1C-F). To determine whether elevation of oTau was correlated to oxidative stress, we quantitatively measured the intracellular reactive oxygen species (ROS) levels in the hippocampus by highly specific electron paramagnetic resonance (EPR) spectroscopy. Intracellular ROS levels as indicated by EPR peaks were significantly elevated in the AD hippocampus (Fig. 1G-H). Furthermore, oTau levels were positively correlated with ROS (Figs. 1I), suggesting a possible link between oTau accumulation and ROS production/accumulation relevant to AD pathology.

**Accumulation of tau oligomers associates with ROS in P301S tauopathy mice**

Next, we determined tau oligomers, ROS levels, and their association with tau pathology in tauopathy mice. Immunostaining revealed increased oTau in the hippocampus and cortex of P301S mice and their presence in cortical neurons (Fig. 2A-C). Compared to age-matched nonTg mice, P301S mice displayed robustly elevated oTau in the hippocampus and entorhinal cortex at 9-months-old, as shown by immunodot blotting (Fig. 2D-E), but not at 1-month-old (Supplementary Fig. 1A and D). Similarly, phosphorylation of tau (Ser202 and Thr205) was significantly elevated in the hippocampus and entorhinal cortex of 9-month old P301S mice (Figs. 2F-G), but not in 1-month-old P301S mice.
The intracellular ROS levels indicated by EPR peaks were significantly elevated in the 9-month-old P301S hippocampus and entorhinal cortex compared to nonTg mice (Figs. 2H-K), but not in 1-month-old P301S mice (Supplementary Fig. 1B and E). Levels of oTau were positively correlated with ROS levels (Figs. 2L-M). These data suggest that tau oligomers are associated with aging and oxidative stress relevant to tau pathology.

**Tau oligomer accumulation correlates with elevated oxidative stress in an Aβ-producing AD mouse model**

Given the potential relationship between abnormal tau accumulation and excessive Aβ and oxidative stress

We assessed the effect of Aβ on tau oligomer levels in Aβ-producing transgenic mice. In mAPP mice at 12 months of age, a timepoint with tremendous accumulation of cerebral Aβ, we observed significant accumulation of oTau staining in the hippocampus (Fig. 3A and B) and entorhinal cortex (Fig. 3A and C). Similarly, immunodot blotting showed, respectively, a ~ 4.5 and ~ 5.5-fold increase of oTau in the hippocampus (Fig. 3D) and entorhinal cortex (Fig. 3E). Furthermore, we studied the progression of oTau deposition by assessing expression of oTau in different ages of mAPP mice. As shown in Fig. 4, oTau were significantly elevated in the hippocampus (Fig. 4A) and the entorhinal cortex (Fig. 4B) of mAPP mice compared to nonTg controls in an age-dependent manner starting at 6–9 months of age, with a greater degree of accumulation at 12–18 months of age, a time of tremendous accumulation of cerebral Aβ. The ROS levels in the hippocampus of mAPP were significantly elevated starting at 6 months of age, prior to the start of tau oligomers accumulation (Fig. 4C-D), and importantly, tau oligomers content was significantly correlated to ROS levels (Fig. 4E-F). These studies indicate the impact of Aβ on tau oligomer accumulation in an Aβ-rich environment.

**Accumulation of tau oligomers is associated with ROS in human MCI and AD cybrids**

Mitochondrial dysfunction is one of the early pathological features of AD. Dysfunctional mitochondria produce excessive ROS. Human trans-mitochondrial “cybrid” (cytoplasmic hybrid) neuronal cells whose mitochondria are derived from platelets of patients with sporadic AD or mild cognitive impairment (MCI) exhibit significant changes in mitochondrial structure and function and increases in ROS generation/accumulation

We therefore utilized MCI and AD cybrids as an ex vivo model to determine the potential impact of MCI- and AD-derived mitochondrial defects on pathological tau oligomers. Levels of tau oligomers were significantly increased in differentiated MCI and AD cybrids compared to non-AD controls. AD cybrid cells exhibited higher levels of tau oligomers than MCI cybrids (Fig. 5A), suggesting that accumulation of tau oligomers is associated with progression of mitochondrial perturbation in AD. Similar results were obtained from MCI and AD platelets (Fig. 5B). ROS levels were also significantly elevated in differentiated AD and MCI cybrids (Figs. 5C) and their derived platelets (Figs. 5D), which was positively correlated with tau oligomer contents (Figs. 5E-F). In addition, treatment with a mitochondria-targeted antioxidant, mito-Tempo, significantly reduced both tau oligomer formation (Fig. 5G) and ROS (Fig. 5H) in MCI and AD cybrids. These results suggest the relevance of increased accumulation of tau oligomers to AD-mediated mitochondrial defects and oxidative stress.
Scavenging mitochondrial ROS eliminates tau oligomers in Aβ and tau neurons in vitro

To further evaluate the contribution of mitochondrial ROS to tau aggregation, Aβ- and tau-producing neurons cultured from mAPP mice and P301S tau mice, respectively, were treated with mito-TEMPO, a scavenger for mitochondria-derived ROS. Compared to nonTg neurons, mAPP and tau neurons display significantly elevated levels of tau oligomers, with TOC1 staining and expression significantly distributed along the cellular bodies and processes; mito-TEMPO treatment strikingly inhibited these TOC1 positive staining signals (Figs. 6A-B). Treatment with mito-TEMPO almost completely reduced tau oligomer levels to those of the vehicle controls as demonstrated by TOC1 immunodot blotting (Fig. 6C). Functionally, mito-TEMPO treatment not only alleviated mitochondrial defects, as shown by increased activity of key mitochondrial respiratory enzymes (complex IV in Fig. 6D and complex I in Fig. 6E) and ATP levels (Fig. 6F), but also suppressed ROS levels (Fig. 6G-H). Similarly, mito-TEMPO abolishment of Aβ- and tau-mediated oTau formation was positively correlated with reduction in ROS levels in Aβ- and Tau-producing neurons (Fig. 6I). These data indicate that scavenging mitochondrial ROS blocks Aβ-, tau- and AD-mediated pathological tau oligomer accumulation and restores mitochondrial function.

Scavenging mitochondrial ROS reduces tau oligomers and improves learning and memory in AD mice

In view of the association between elevation of tau oligomers and oxidative stress in Aβ-producing AD mice (Fig. 4), we assessed the effects of Aβ-mediated mitochondrial defects and excessive ROS on tau oligomer accumulation and cognitive function (Fig. 7). mAPP mice overexpressing Aβ were treated with mito-TEMPO starting at 6–7 months of age for three months and then analyzed for tau oligomers (Fig. 7A), ROS (Fig. 7B), mitochondrial function (Fig. 7C-D) and behavioral endpoints (Fig. 7E-I). Mito-TEMPO treatment abolished accumulation of tau oligomers, as shown by immunodot blotting, and ROS, as shown by EPR measurement, in the entorhinal cortex of mAPP mice, as compared to vehicle treatment (Fig. 7A-B). Furthermore, mAPP mice treated with mito-TEMPO displayed significantly increased respiratory chain complex IV activity and ATP levels (Fig. 7C-D) and improved learning and memory as evidenced by decreased escape latencies, increased number of target crossings, and increased time spent in the target quadrant in the Morris Water Maze task (MWM, Fig. 7E-I). These results suggest the protective effect of eliminating Aβ-involved mitochondrial ROS on pathological tau oligomers accumulation, mitochondrial perturbation, and impairment in learning and memory.

Discussion

Tauopathies are a class of neurodegenerative disorders characterized by hyperphosphorylation and aggregation of the microtubule-associated protein tau (MAPT) into paired helical filaments (PHFs) or straight filaments (SFs), leading to the formation of neurofibrillary tangles (NFTs). Tau monomers bind to each other to form oligomeric tau when hyperphosphorylated tau dislodges from microtubules. Tau oligomers potentiate neuronal damage, leading to traumatic brain injury and neurodegeneration 19–21. Recent studies have demonstrated that tau oligomers are responsible for progression of tau pathology 6. Up-regulated granular tau oligomer levels occur prior to NFT formation and clinical symptoms of AD.
Reduction of tau by doxycycline treatment improved memory impairment in P301L tau mice without affecting NFT formation, suggesting an early role for tau oligomers in AD pathogenesis relevant to cognitive decline. However, the causes and co-stimulating factors that enhance pathological tau formation and accumulation relevant to the pathogenesis of AD remain largely unknown.

In the present study, we analyzed levels of tau oligomers in the human AD hippocampus, AD-related Aβ and tauopathy mouse models, and MCI- and AD-derived mitochondria. Levels of tau oligomers were significantly elevated in AD brains, Aβ and tau overexpressed mice, and in vitro in Aβ and tau neurons. Interestingly, tau oligomers accumulated in an age-dependent manner in mAPP mice, with significant elevation starting at 6–7 months of age, a time point corresponding to the appearance of behavioral abnormalities in mAPP mice. Our findings are consistent with a previous study, which revealed that brief exposure to extracellular recombinant human tau oligomers, rather than monomers or Aβ, results in impairment in long-term potentiation (LTP) and learning and memory.

Overproduction of ROS and oxidative stress-mediated cellular perturbation are known to be key players in AD pathogenesis, including tauopathy. However, it is the causes and consequences of pathological tau metabolism such as toxic tau oligomers remain unclear. We observed that accumulation of tau oligomers is significantly elevated and positively correlated to ROS levels in human AD brains, MCI- and AD-derived mitochondria, and amyloid and tauopathy mice. Importantly, scavenging mitochondrial ROS by treatment with mito-TEMPO, a mitochondria-targeted antioxidant, strikingly reduced tau oligomers accumulation in cultured tau neurons. Intriguingly, mito-TEMPO also suppressed Aβ- and AD mitochondria-induced tau oligomers accumulation and rescued mitochondrial respiratory function. In Aβ-producing AD mice, treatment with mito-TEMPO significantly diminished tau oligomers and improved cognition. These results indicate the contribution of mitochondrial ROS to tau oligomer formation and accumulation. Given that Aβ oligomers can seed and promote tau oligomerization and uptake of tau fibrils, we propose that Aβ-mediated sustained mitochondrial stress and excessive ROS production could be a potential mechanism underlying Aβ-mediated/enhanced tau oligomers accumulation. Our studies suggest the role of the Aβ/ROS/mitochondria axis in aberrant tau oligomer accumulation, which links to cognitive dysfunction. The detailed mechanisms require further investigation in the near future. It is noted that tau oligomers were not significantly increased in AD-spared regions such as the cerebellum when compared to non-AD controls, nor were there significant changes in ROS levels in these spared regions (data not shown). Thus, accumulation of tau oligomers is associated with AD-pathology.

Taken together, using multiple AD models, we have provided substantial evidence of the connection between mitochondrial ROS and the accumulation of tau oligomers. Blocking mitochondrial oxidative stress eliminates accumulation of tau oligomers and improves mitochondrial function relevant to amyloid and tau pathology. Thus, our findings support the possibility that inhibiting mitochondrial oxidative stress and dysfunctions could be a promising therapeutic target to prevent and treat tauopathies.
Materials And Method

**Animal studies.** Animal studies were carried out with the approval of the Institutional Animal Care and Use Committee of the University of Kansas Lawrence and Columbia University in New York in accordance with the National Institutes of Health guidelines for animal care.

**Human subjects.** We obtained hippocampal and cerebellar tissues from individuals with Alzheimer's disease and age-matched, non-Alzheimer's disease controls from the New York Brain Bank at Columbia University. Detailed information for each of the cases studied is shown in Supplementary Table 1 online. Informed consent was obtained from all subjects.

**Primary neuronal culture**

Hippocampal neurons from day 1 nonTg, mAPP, or P301S tau mice were prepared as described previously. Neurons were cultures in neurobasal medium supplemented with 1 x B27, 600 μM L-Glutamine and penicillin-streptomycin. At day 14 *in vitro* (DIV), neurons from the indicated mice were used for experiments.

**Evaluation of intracellular reactive oxygen species (ROS)**

Evaluation of intracellular ROS levels was conducted by electron paramagnetic resonance (EPR) spectroscopy. Brain tissues or cultured neurons was incubated with CMH (cyclic hydroxylamine 1-hydroxy-3-methoxycarbonyl-2, 2, 5, 5-tetramethyl-pyrrolidine, 100 μM) for 30 minutes and then washed three times with cold PBS. Subsequently, brain tissues and neurons were collected and homogenized with 100 μl of PBS for EPR measurement. The EPR spectra were recorded, stored, and analyzed with a Bruker EleXsys 540 X-band EPR spectrometer (Billerica, MA) using Bruker Xepr software Xepr (Billerica, MA).

**Measurement of respiratory chain complex activities and ATP levels**

Mitochondrial respiratory complex I activities were measured in neuronal homogenates as described previously. NADH:ubiquinone oxidoreductase (COX I) enzyme activity was determined in 25 mM potassium buffer containing KCl, Tris-HCl and EDTA (pH 7.4). Homogenized samples (50 μg protein) were incubated with with 2 μg/ml antimycin, 5mM MgCl₂, 2mM KCN and 65 μM co-enzyme Q1 were and the oxidation of NADH was recorded for 3 min. Subsequently, 2 μg /ml rotenone was added and the absorbance was measured for another 3 min. The change in absorbance was monitored at 340 nm using an Amersham Biosciences Ultrospect 3100 Pro spectrophotometer.

Cytochrome c oxidase (CcO, complex IV) activity was spectrophotometrically determined using the Cytochrome c Oxidase Assay Kit (Sigma) as described in our previous study. In brief, neurons were collected using lysis buffer, incubated on ice for 15 minutes, and centrifuged at 12,000g for 10 minutes. Suitable volumes of lysates and enzyme solutions were added into 475 μl of assay buffer. The reaction was triggered by the addition of 25 μl of ferrocytochrome c substrate solution (0.22 mM). Changes in
absorbance of cytochrome c at 550 nm were immediately recorded using a kinetic program with 5 second delay, 10 second intervals, for a total of 6 readings on an Ultrospect 3100 Pro spectrophotometer.

ATP levels were determined using an ATP Bioluminescence Assay Kit (Roche) following the manufacturer’s instruction. Briefly, neurons were collected in the provided lysis buffer, incubated on ice for 30 minutes, and centrifuged at 12,000g for 10 minutes. ATP levels were then measured in the subsequent supernatants using a luminescence plate reader (Molecular Devices). A 1.6 second delay after substrate injection and 10 seconds integration time were used.

**Immunoblotting analysis**

Brain tissue lysates for immunoblotting were prepared following the method described in our previous study. Protein lysates were subjected to 10% Bis-Tris gel electrophoresis (Invitrogen, Grand Island, NY, USA), transferred to nitrocellulose membrane, incubated with 5% non-fat dry milk in TBST buffer (20 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20) for 1 hour at room temperature, followed by incubation with primary antibodies under gentle agitation overnight at 4°C. The following primary antibodies were used: Toc1 (oligomeric tau, mouse IgM, provided by Dr. Kanaan (Nicholas M. Kanaan, Department of Translational Neuroscience, Michigan State University), AT8: anti-phospho-Tau pSer202/Thr205 (MN1020, Thermo Fisher Scientific), and β-actin (A5441; Sigma-Aldrich). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for quantification of the intensity of the immunoreactive bands in the developed blots.

**Immunodot blotting**

Brain or cell lysates were prepared and analyzed as described for immunoblotting with the following modifications. Samples were spotted onto the nitrocellulose membrane using a Whatman Minifold I immunodot blotting apparatus. The membranes were blocked, and probed with TOC1 and β-actin (A5441; Sigma-Aldrich). Signal intensity measurements for each dot were expressed as the ratio of oligomeric tau (TOC1 signal) to β-actin. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for quantification of the intensity of the developed blots.

**Immunohistochemical staining**

Brain slices from the indicated Tg mice were subjected to double immuno-staining with anti-Toc1 (oligomeric tau, mouse IgM) and mouse anti-MAP2 (1:5000, sc-33796, Santa Cruz Biotechnology) at 4°C overnight followed by Alexa Fluor 488 and 594 goat anti-mouse, respectively. Images were acquired on a Leica SP5 confocal microscope and analyzed using Leica LAS AF software (Leica Wetzlar) and MetaMorph (Molecular Devices) Program.

**Behavioral Test**

The Morris Water Maze (MWM) test was performed according to the method described in previous publications. During the spatial acquisition session, mice were trained for 6 consecutive days with 4
trials per mouse per day. Maximum time for each trial was capped at 60 s. Escape latency was analyzed by the HVS Image software (2015). On day 7, a probe trial was performed to assess the spatial memory of the mice. The platform was removed from the pool and the mice were allowed to swim freely for 60 s. Traces of the mice swim paths were recorded and analyzed by HVS Image. Investigators were blind to mouse genotypes and treatment groups.

**Statistical Analysis.** All data were expressed as the mean ± SEM. Student t-tests were performed for analysis and comparisons between two groups. Data were analyzed by one-way ANOVA for repeated measure analysis using commercially available software (Statview, version 5.0.1, Berkeley, Calif), followed by Fisher’s protected least significant difference for post hoc comparisons. P < 0.05 was considered significant.

**Declarations**

**Acknowledgement:** This study was supported by NIH/NIA (R37AG037319, R01AG044793, R01AG054320, R01AG05304, and R56AG053041), and Alzheimer's Association Research Grant (AARG, 2018-AARG-592230 Alzheimer's Association). We thank Dr. Kanaan for providing the TOC1 antibody, and Justin T. Douglas for assistance using the EPR instrument. The EPR instrumentation was provided by NSF Chemical Instrumentation Grant (# 0946883).

**Author contributions:** S.S.Y. initiated and supervised the research, designed experiments, developed the concept and wrote the paper. F.D. designed and performed experiments, analyzed data and wrote the paper. Q.Y. performed experiments on mitochondrial function, immunoblotting, behavior and analyzed data. Doris Chen performed characterization of mouse genotype and edited manuscript. S.F.Y. edited the manuscript.

**Conflict of Interest statement:** None declared.

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**Figures**
Figure 1

Tau oligomers pathology and ROS levels in human Alzheimer’s disease (AD) hippocampus. (A-B) Tau Oligomeric Complex 1 (TOC1) immunohistochemistry on AD hippocampus. (A) Representative images showed TOC1 immunohistochemistry on hippocampus (I: ND and II: AD; TOC1: Green, MAP2: Red). (B) Quantification of TOC1 immunohistochemistry was performed with the hippocampus from the indicated hippocampal sections. N = 4 per group. (C-F) The graph presents quantification of immunodot blotting (D...
and F) for TOC1 normalized to β-actin on hippocampus (C-D) and cerebellum (E-F) of ND and AD brains. N = 7 per group. The representative immunodot blotting for TOC1 from indicated hippocampal (C and D) and cerebellum (E and F) homogenates, and β-actin served as a loading control. (G-H) Quantification of EPR (G) and representative spectra of EPR spectra (H) in the indicated hippocampal homogenous. The peak height in the spectrum indicates levels of ROS. N = 7 per group. (I) Correlation analysis of the relationship between TOC1 and ROS levels on hippocampus. N = 14 per correlation analysis. Scale bar = 20 µm.

**Figure 2**
Figure 2

Tau oligomers pathology and ROS levels in 9-month-old P301S mutant human tau transgenic mice. (A-C) TOC1 immunohistochemistry on hippocampus and entorhinal cortex from 9-month-old P301S mutant human tau transgenic mice. (A) Representative images showed TOC1 immunohistochemistry on hippocampus (I and II) and entorhinal cortex (III and IV). I, III: nonTg, and II, IV: P301S Tau transgenic mice (TOC1: Green, MAP2: Red). (B-C) Quantifications of TOC1 immunohistochemistry were performed in the hippocampus (B) and entorhinal cortex (C) from the indicated mice. N = 3 mice per group. (D-E) The bar graph presents quantification of immunodot blotting bands for TOC1 normalized to β-actin on hippocampus (D) and entorhinal cortex (E) of the indicated mice. N = 3 mice per group. The representative immunodot blotting for TOC1 were shown from indicated hippocampal (D) and entorhinal cortex (E) homogenates, and β-actin served as a loading control. (F-G) The bar graph presents quantification of immunoreactive bands for AT-8 normalized to β-actin on hippocampus (F) and entorhinal cortex (G) of the indicated mice. N = 3 mice per group. The representative immunoblots show immunoreactive bands for AT-8 from indicated hippocampal (F) and entorhinal cortex (G) homogenates, and β-actin served as a loading control. (H-K) Quantification of EPR spectra (H: hippocampus and I: entorhinal cortex) and representative spectra of EPR (J: hippocampus and K: entorhinal cortex) in the indicated mice. The peak height in the spectrum indicates levels of ROS. N = 3 mice per group. (L-M) Correlation analysis of the relationship between TOC1 and ROS levels on hippocampus (L) and entorhinal cortex (M). N = 6 mice per correlation analysis. Scale bar = 50 µm.
Figure 3

Tau oligomers pathology in 12-month-old mAPP mice. (A-C) TOC1 immunohistochemistry on hippocampus and entorhinal cortex from 12-month-old mAPP mice. (A) Representative images showed TOC1 immunohistochemistry on hippocampus (I and II) and entorhinal cortex (III and IV). I, III: nonTg, and II, IV: mAPP transgenic mice (TOC1: Green, MAP2: Red). (B-C) Quantifications of TOC1 immunohistochemistry was performed with the hippocampus (B) and entorhinal cortex (C) from the
indicated mice. N = 3 mice per group. (D-E) The bar graph presents quantification of immunodot blotting for TOC1 normalized to β-actin on hippocampus (D) and entorhinal cortex (E) of the indicated mice. N = 3 mice per group. The representative immunodot blotting for TOC1 from indicated hippocampal (D) and entorhinal cortex (E) homogenates, and β-actin served as a loading control. Scale bar = 50 µm.
Age-dependent Tau oligomers pathology and ROS levels in mAPP mice. (A-B) The bar graph presents quantification of immunodot blotting for TOC1 normalized to β-actin on hippocampus (A) and entorhinal cortex (B) of the 3, 6, 9, 12 and 18-month-old mAPP mice. N = 3 mice per group. The representative immunodot blotting for TOC1 from indicated hippocampal (A) and entorhinal cortex (B) homogenates, and β-actin served as a loading control. (C-D) Quantification of EPR spectra (C) and representative spectra of EPR (D) in the hippocampus and entorhinal cortex from the indicated mice. The peak height in the spectrum indicates levels of ROS. N = 3 mice per group. (E-F) Correlation analysis of the relationship between TOC1 and ROS levels on hippocampus (E) and entorhinal cortex (F). N = 30 mice per correlation analysis.
Figure 5

Figure 5

Tau oligomers pathology and ROS levels in differentiated AD and mild cognitive impairment (MCI) trans-mitochondrial Cybrid cells and human platelets. (A-B) The bar graph presents quantification of immunodot blotting for TOC1 normalized to β-actin on differentiated MCI and AD trans-mitochondrial Cybrid cells (A) and human platelets (B). N = 5 for Cybrids and 7 for platelets per group. The representative immunodot blottings were shown for TOC1 from indicated cybrids (A) and platelets (B),
and β-actin served as a loading control. (C-D) Quantifications of EPR spectra and representative spectra of EPR in the indicated cybrids (C) and platelets (D). The peak height in the spectrum indicates levels of ROS. N = 5 for cybrids and 7 for platelets per group. (E-F) Correlation analysis of the relationship between TOC1 and ROS levels on Cybrids (E) and platelets (F). N = 15 for Cybrids and 21 for platelets per correlation analysis. (G) The bar graph presents quantification of immunodot blotting for TOC1 normalized to β-actin on differentiated MCI and AD trans-mitochondrial Cybrid cells with or without mito-TEMPO treatment. The representative immunodot blottings were shown for TOC1 from indicated cybrids and β-actin served as a loading control. (H) Quantifications of EPR spectra and representative spectra of EPR in the indicated cybrids. N = 5 for Cybrids per group.
**Figure 6**

Effect of Mito-TEMPO on Tau oligomers pathology and ROS levels in mAPP and TAU neurons in vitro. (A-B) TOC1 immunocytochemistry 21 days in vitro (DIV) hippocampal neurons. (B) Representative images showed TOC1 immuncytochemistry on hippocampal neurons (TOC1: Green, MAP2: Red). I: nonTg, II: mAPP, III: mAPP + TEMPO, IV: TAU, and V: TAU + TEMPO. The right panels are the enlarged views of TOC1 in the left panels. Quantification of TOC1 immunocytochemistry in A. N = 8 neurons per group. (C) The
bar graph presents quantifications of immunodot blottings (C) for TOC1 normalized to β-actin of the indicated neurons. N = 3 per group. The representative immunodot blottings (C) for TOC1 were shown from indicated neurons, and β-actin served as a loading control. (D-F) Complex IV (D) and I (E) activities and ATP levels (F) were determined in indicated groups. Data are expressed as fold change relative to the vehicle group (n = 3 independent experiments). (G-H) Quantification of EPR (G) and representative spectra of EPR spectra (H) in the indicated hippocampal neurons. The peak height in the spectrum indicates levels of ROS. N = 4 per group. (I) Correlation analysis of the relationship between TOC1 and ROS levels on hippocampal neurons. N = 15 per correlation analysis. *P < 0.01 vs. vehicle groups in A, C, D, E, F and G. Scale bars = 25 μm.
Figure 7

Mito-TEMPO rescues Tau oligomers pathology and ROS levels in AD mice in vivo. (A) The bar graph presents quantification of immunodot blotting for TOC1 normalized to β-actin in the entorhinal cortex of 6-7-month old mAPP mice with or without mito-TEMPO treatment for 3 months. (B) Quantifications of EPR spectra and representative spectra of EPR in the indicated mice. N = 4-6 mice per group. (C-D) Complex IV (C) activity and ATP levels (D) were determined in the entorhinal cortex from the indicated
mice groups. Data are expressed as fold change relative to the vehicle group (n = 3 independent experiments). (E-I) Effect of mito-TEMPO on learning and memory in mAPP mice. Learning and memory were tested using the Morris water maze (MWM) in the indicated groups of mice. (E) Mean escape latency to the hidden platform during each day of the training session. *P < 0.01 compared to nonTg and mAPP mice. (F) Pattern of representative searching traces for the indicated mice in search of the target. (G) Mean number of crossings of the target during probe trials. (H) Time spent in the target area in probe trials, and (I) The speed in the indicated groups has no difference.

Supplementary Files

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- sdy782020Supplementary.doc