Published in final edited form as:

Mol Psychiatry. 2021 February ; 26(2): 586–603. doi:10.1038/s41380-018-0286-z.

δ -Secretase-cleaved Tau stimulates A β production via upregulating STAT1-BACE1 signaling in Alzheimer's disease

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Abstract

δ -Secretase, an age-dependent asparagine protease, cleaves both amyloid precursor protein (APP) and Tau and is required for amyloid plaque and neurofibrillary tangle pathologies in Alzheimer's disease (AD). However, whether δ -secretase activation is sufficient to trigger AD pathogenesis remains unknown. Here we show that the fragments of δ -secretase-cleavage, APP (586–695) and Tau(1–368), additively drive AD pathogenesis and cognitive dysfunctions. Tau(1–368) strongly augments BACE1 expression and A β generation in the presence of APP. The Tau(1–368) fragment is more robust than full-length Tau in binding active STAT1, a BACE1 transcription factor, and promotes its nuclear translocation, upregulating BACE1 and A β production. Notably, A β -activated SGK1 or JAK2 kinase phosphorylates STAT1 and induces its association with Tau(1–368). Inhibition of these kinases diminishes stimulatory effect of Tau(1–368). Knockout of STAT1 abolishes AD pathologies induced by δ -secretase-generated APP and Tau fragments. Thus, we show that Tau may not only be a downstream effector of A β in the amyloid hypothesis, but also act as a driving force for A β , when cleaved by δ -secretase.

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Electronic supplementary material The online version of this article (<https://doi.org/10.1038/s41380-018-0286-z>) contains supplementary material, which is available to authorized users.

Conflict of interest The authors declare that they have no conflict of interest.

Introduction

Alzheimer's disease (AD) is the major cause of dementia in the elderly, leading to memory loss and cognitive decline. It is characterized by the extracellular accumulation of β -amyloid peptide ($A\beta$) within the brain, along with intraneuronal hyperphosphorylated and cleaved forms of the microtubule-associated protein, Tau. It is known that a dysfunctional metabolism of amyloid precursor protein (APP) and the abnormal Tau protein phosphorylation lead to the formation of neuritic plaques and neurofibrillary tangles (NFT), respectively. Misfolded and aggregated proteins bind to pattern recognition receptors on microglia and astroglia, and trigger an innate immune response characterized by the release of inflammatory mediators, which contribute to disease progression and severity [1]. These events finally drive neuronal cell death and the clinical expression of dementia.

Very recently, δ -secretase has been shown to cleave APP on its extracellular motif in AD brains, at both N373 and N585 sites. The resultant C-terminal fragment APP(586–695) is a better substrate for BACE1 to generate $A\beta$ than APP full-length (FL) [2]. APP(586–695) also cofractionates with APP in the early endo-lysosome, where BACE1 resides [2]. In addition, δ -secretase cleaves Tau at N255 and N368 residues in AD brains and this cleavage enhances Tau aggregation. The Tau(1–368) fragment is very neurotoxic [3]. Of particular interest, δ -secretase expression levels and activities are escalated during ageing, cleaving both APP and Tau. Knockout of δ -secretase in 5XFAD or Tau P301S mouse models of AD significantly abrogates AD pathologies and rescues the cognitive impairments. Thus, δ -secretase activity is necessary for the major features of AD pathogenesis [2, 3]. δ -secretase acts as an asparagine endopeptidase (AEP), which usually localizes in the lysosomes and functions as a scavenger. δ -secretase is a pH-regulated protease that is mainly activated by acidosis [4, 5]. Once activated, it predominantly cleaves the substrates after asparagine residues [6]. Previously, we have reported that δ -secretase cuts SET, a nuclear protein inhibiting DNase, leading to DNA nicking and neuronal cell death during stroke. Depletion of δ -secretase substantially blocks neuronal loss induced by ischemia [7]. Transient occlusion of the middle cerebral artery (MCAO) initiates AEP upregulation in the peri-infarct area of rats, and depletion of δ -secretase reduces CD74+ cells. Thus, δ -secretase is involved in mechanisms of post-stroke tissue inflammation and may contribute to an invasion of inflammatory cells toward the ischemic core [8]. The results of all of these studies are consistent with the notion that δ -secretase mediates the formation of amyloid plaque and NFTs, neuro-inflammation and neuronal cell death, which are the major pathological features in AD.

According to the amyloid hypothesis, accumulation of $A\beta$ in the brain is the primary influence driving AD pathogenesis. The rest of the disease process, including the formation of NFT containing Tau protein, is proposed to result from an imbalance between $A\beta$ production and $A\beta$ clearance [9]. This hypothesis, which also posits that the deposition of $A\beta$ in the brain is the cause of neural/synaptic damage and dementia, has dominated AD research for more than two decades [10]. The main evidence in support of this hypothesis is that all dominant mutations causing early-onset AD occur either in the substrate (APP) or the protease (presenilin) of the reaction that generates $A\beta$ [11]. Duplication of wild-type APP gene in Down's syndrome leads to $A\beta$ deposits in the teens [12]. Moreover,

Apolipoprotein E4 (APOE4), which predisposes to AD in >40% of cases, has been found to impair A β clearance from the brain [13]. Furthermore, soluble A β oligomers can inhibit hippocampal long-term potentiation (LTP) in rats, and disrupts memory when injected to young rats [14, 15]. Lastly, the human oligomers also induce hyperphosphorylation of Tau at AD-relevant epitopes and cause neuritic dystrophy in cultured neurons [16].

However, mounting evidence challenges this over-simplified one-way amyloid-Tau degenerative cascade. For instance, many cognitively normal elderly subjects have relatively large amounts of A β in their brains [17–19]. Recently, imaging A β with Pittsburgh compound B and florbetapir PET studies in cognitively normal subjects showed that almost a third of elderly individuals have major amounts of A β in their brain [20, 21]. So far, the clinical trials with therapeutics that aim to reduce the levels of amyloid- β in the brain all have failed [22], which leads to questions on the role of A β in AD [23, 24]. Furthermore, intraneuronal Tau alterations precede aggregated A β deposition in Braak-stage 1–3 in the pre-symptomatic stages of AD [25], and Tau tangles develop temporally either before or independent of A β plaques [26–28]. Moreover, in double transgenic mice expressing both human mutant Tau and APP, greater A β deposition and NFT-like formation with increased neuronal loss is found when compared with APP or Tau single transgenic mice, indicating that Tau may accelerate amyloid deposition [29]. Most recently, it has been reported that human secreted Tau increases A β production in human neurons [30]. Together, these divergent findings indicate that the causal link between aberrant APP processing and Tau alterations remains controversial.

In the current study, we provide compelling evidence demonstrating how δ -secretase-cleaved Tau(1–368) fragment facilitates A β production. We show that the Tau(1–368) fragment selectively binds active STAT1 (the signal transducers and activators of transcription), a BACE1 transcription factor [31, 32], in the nucleus, and upregulates BACE1 and subsequent A β generation, leading to AD pathologies. Tau(1–368) strongly escalates BACE1 levels in the presence of APP or A β , which is dependent on p-STAT1 induced by A β -activated SGK1 or JAK2 kinase. Inhibition of these kinases or β/γ -secretases diminishes activity of Tau(1–368). Deficiency of STAT1 also abrogates the effect of Tau(1–368) on BACE1. Hence, our findings provide an innovative insight into the molecular mechanisms of how Tau feeds back and regulates A β upon cleavage by δ -secretase.

Materials and methods

Mice

Male C57BL/6J mice and 3 $\times$ Tg mice were from Jackson Laboratory (stock number: 000664, 34830, respectively). Animal care and handling was performed according to the Declaration of Helsinki and Emory Medical School guidelines. Sample size was determined by Power and Precision (Biostat). The animals were randomly allocated to experimental groups. Investigators were blinded to the group allocation during the animal experiments. The protocol was reviewed and approved by the Emory Institutional Animal Care and Use Committee.

Human tissue samples

Post-mortem brain slides of AD subjects with different Braak stage were from the Emory Alzheimer's Disease Research Center. AD was diagnosed according to the criteria of the Consortium to Establish a Registry for AD and the National Institute on Aging. Diagnoses were confirmed by the presence of amyloid plaques and NFT in formalin-fixed tissue. The average age was 74.89 ± 12.0 (mean $\pm$ s. d.), The post-mortem interval was similar between groups. The study was approved by the biospecimen committee at Emory University. Informed consent was obtained from all subjects.

Transfection and infection of the cells

HEK293 cells were transfected with plasmids encoding GFP, APP full-length, APP(1–373), APP(1–585), APP (586–695), Tau full-length, TauN388, STAT1, JAK2, SGK1 using Polyethylenimine. Lentivirus were used to express wild-type and mutant δ -secretase in primary neurons.

δ -secretase activity assay

Tissue homogenates or cell lysates (10 μ g) were incubated in 200 μ l assay buffer (20 mM citric acid, 60 mM Na_2HPO_4 , 1 mM EDTA, 0.1% CHAPS, and 1 mM DTT, pH 6.0) containing 20 μ M δ -secretase substrate Z-Ala-Ala-Asn-AMC (Bachem). AMC released by substrate cleavage was quantified by measuring at 460 nm in a fluorescence plate reader at 37 °C for 1 h in kinetic mode for 5 min.

Western blot analysis

The mouse brain tissue or human tissue samples were lysed in lysis buffer (50 mM Tris, pH 7.4, 40 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 1.5 mM Na_3VO_4 , 50 mM NaF, 10 mM sodium pyrophosphate, 10 mM sodium β -glycerophosphate, supplemented with a cocktail of protease inhibitors), and centrifuged for 15 min at $16,000 \times g$. The supernatant was boiled in SDS loading buffer. After SDS-PAGE, the samples were transferred to a nitrocellulose membrane. Primary antibodies to the following targets were used: GST-HRP, α -tubulin, and β -actin (Sigma-Aldrich), HA and GFP (Santa Cruz Biotechnology), BACE1, STAT1, p-STAT1, JAK2, p-JAK2, and p-SGK1 (cell signaling), SGK1 (Abcam), HT7 and AT8 (Thermo Fisher), δ -secretase antibody (clone 6E3, president from Dr. Colin Watts, University of Cambridge).

Immunostaining

Paraffin embedded human brain sections or free-floating mouse brain sections were treated with 0.3% H_2O_2 for 10 min. Sections were thereafter washed three times in PBS and blocked in 1% BSA, 0.3% Triton X-100, for 30 min followed by overnight incubation with primary antibodies at 4 °C. The primary antibodies include: HT7 (Thermo Fisher, 1: 1000), AT8 (Thermo Fisher, 1:500), 4G8 (Covance, 1:500), NeuN (cell signaling, 1:1000). The signal was developed using Histostain-SP kit (Invitrogen). To detect the colocalization of Tau(1–368), $\text{A}\beta$, and STAT1 in human PD brain sections, the sections were incubated with anti-Tau(1–368), anti- $\text{A}\beta$, and anti-STAT1 antibody overnight at 4 °C. The slides were washed three times in PBS, and incubated with Texas Red-conjugated anti-mouse IgG and

FITC-conjugated anti-rabbit IgG for 1 h at room temperature. The slides were washed three times in PBS, then covered with a glass cover using mounting solution and examined under a fluorescence microscope (Olympus).

Co-IP and GST pull-down

The mouse brain tissue samples were lysed in lysis buffer (50 mM Tris, pH 7.4, 40 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 1.5 mM Na₃VO₄, 50 mM NaF, 10 mM sodium pyrophosphate, 10 mM sodium β -glycerophosphate, supplemented with a cocktail of protease inhibitors) and centrifuged for 15 min at $16,000 \times g$. The supernatant was incubated with HT7 antibody and protein A/G-agarose overnight at 4 °C. After extensive washing, the bound proteins were eluted from the beads by boiling in Laemmli sample buffer and subjected to western blot analyses. For GST pull-down assay, cell lysis was incubated with Glutathione Sepharose beads (GE healthcare) overnight and washed extensively. The bound proteins were eluted from the beads by boiling in Laemmli sample buffer and subjected to western blot analyses.

Extraction of nuclear fraction

HEK293 cells were collected and washed with PBS. The cells were gently resuspended and incubated in 500 μ l hypotonic buffer (20 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂) on ice for 15 min. Twenty-five microliters of 10% NP40 was added and vortexed for 10 s to break down the nuclei. The homogenate was centrifuged for 10 min at 3000 rpm at 4 °C. The pellet was resuspended in 50 μ l cell lysis buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10% glycerol, 0.1% SDS, 1.5 mM Na₃VO₄, 50 mM NaF, 0.5% deoxycholate, 10 mM sodium pyrophosphate, 10 mM sodium β -glycerophosphate, supplemented with a cocktail of protease inhibitors) for 30 min on ice with vortexing at 10 min intervals, and then centrifuge for 30 min at $14,000 \times g$ at 4 °C. The supernatant containing nuclear fraction was collected.

A β plaque histology

Amyloid plaques were stained with Thioflavin-S. Free-floating 40- μ m brain sections were incubated in 0.25% potassium permanganate solution for 20 min, rinsed in distilled water and incubated in bleaching solution containing 2% oxalic acid and 1% potassium metabisulfite for 2 min. After rinsed in distilled water, the sections were transferred to blocking solution containing 1% sodium hydroxide and 0.9% hydrogen peroxide for 20 min. The sections were incubated for 5 s in 0.25% acidic acid, then washed in distilled water and stained for 5 min with 0.0125% Thioflavin-S in 50% ethanol. The sections were washed with 50% ethanol and placed in distilled water. Then the sections were covered with a glass cover using mounting solution and examined under a fluorescence microscope. The plaque number and plaque area were calculated using ImageJ software (National Institutes of Health).

Morris water maze

The mice were trained in a round, water-filled tub (52 inch diameter) in an environment rich with extra maze cues. An invisible escape platform was located in a fixed spatial location 1

cm below the water surface independent of a subject's start position on a particular trial. In this manner, subjects needed to utilize extra maze cues to determine the platform's location. At the beginning of each trial, the mouse was placed in the water maze with their paws touching the wall from one of four different starting positions (N, S, E, and W). Each subject was given four trials per day for 5 consecutive days with a 15-min inter-trial interval. The maximum trial length was 60 s and if subjects did not reach the platform in the allotted time, they were manually guided to it. On reaching the invisible escape platform, subjects were left on it for an additional 5 s to allow for survey of the spatial cues in the environment to guide future navigation to the platform. After each trial, subjects were dried and kept in a dry plastic holding cage filled with paper towels to allow the subjects to dry off. The temperature of the water was monitored every hour so that mice were tested in water that was between 22 and 37 °C. Following the 5 days of task acquisition, a probe trial was presented during which time the platform was removed and the percentage of time spent in the quadrant, which previously contained the escape platform during task acquisition was measured over 60 s. All trials were analyzed for latency and swim speed by means of MzeScan (Clever Sys, Inc.).

Electron microscopy

Synaptic density was determined by electron microscopy. After deep anesthesia, mice were perfused transcardially with 2% glutaraldehyde and 3% paraformaldehyde in PBS. Hippocampal slices were postfixed in cold 1% OsO₄ for 1 h. Samples were prepared and examined using standard procedures. Ultrathin sections (90 nm) were stained with uranyl acetate and lead acetate, and viewed at 100 kV in a JEOL 200CX electron microscope. Synapses were identified by the presence of synaptic vesicles and postsynaptic densities. During double blind quantification, electron micrographs (100 μm² CA1 area at 5000×) were digitally zoomed up to 20,000× magnification by the Preview program in a Mac computer as described previously [33].

ELISA quantification of Aβ

To detect the concentration of Aβ in total brain lysates, the mouse brains were homogenized in 8 × mass of 5 M guanidine HCl/50 mM Tris-HCl (pH 8.0), and incubated at room temperature for 3 h. Then the samples were diluted with cold reaction buffer (PBS with 5% BSA and 0.03% Tween-20, supplemented with protease inhibitor cocktail), and centrifuged at 16,000 × g for 20 min at 4 °C. The Aβ in the total brain were determined using human Aβ₄₂ ELISA kit (Thermo Fisher Scientific, Catalog number KHB3441) and human Aβ₄₀ ELISA kit (Thermo Fisher Scientific, Catalog number KHB3481) according to the manufacturer's instructions. The Aβ concentrations were determined by comparison with the standard curve. The Aβ concentrations in the cell culture medium was determined using mouse Aβ₄₀ ELISA kit (Thermo Fisher Scientific, Catalog number KMB3481) according to the manufacturer's instructions.

Golgi staining

Mouse brains were fixed in 10% formalin for 24 h, and then immersed in 3% potassium bichromate for 3 days in the dark. The solution was changed each day. Then the brains were transferred into 2% silver nitrate solution and incubated for 24 h in the dark. Vibratome

sections were cut at 60 μm , air dried for 10 min, dehydrated through 95 and 100% ethanol, cleared in xylene and coverslipped.

Lentivirus and AAVs packaging and stereotaxic injection

The packaging of lentivirus and AAVs and stereotaxic injection was performed as previously described [2, 3]. Briefly, APP full-length and fragments were subcloned into pFCGW-N1 vector, while Tau full-length and Tau(1–368) fragment were subcloned into pAAV vector with a human synapsin I promoter. For stereotaxic injection, 3-month-old wild-type C57BL/6J mice were anesthetized with phenobarbital (75 mg kg⁻¹). Unilateral intracerebral injection of virus was performed stereotaxically at coordinates anteroposterior (AP) –3.1 mm and mediolateral (ML) –1.2 mm relative to bregma, and dorsoventral (DV) –4.0 mm from the dural surface. Two microliters of viral suspension containing 2×10^{11} vector genomes (vg)/ml was injected into each site using 10 μl glass syringes with a fixed needle at a rate of 0.25 $\mu\text{l min}^{-1}$. The needle remained in place for 5 min before it was removed slowly (over 2 min). The mice were placed on a heating pad until they turned from a supine to a prone position, indicative of beginning to recover from the anesthesia.

Statistical analysis

Statistical analysis was performed using either Student's *t*-test (two-group comparison) or one-way ANOVA followed by LSD post hoc test (more than two groups), and differences with *P* values less than 0.05 were considered significant.

Results

δ -secretase-cleaved APP(586–695) fragment induces amyloid plaques, synaptic impairments, and cognitive defects

To test whether δ -secretase cleavage of APP is sufficient to induce amyloid plaque pathology, we injected lentiviral vectors encoding either GFP, GFP-tagged APP FL, or proteolytic fragments of GFP-tagged APP that had been cleaved by δ -secretase into the hippocampal CA1 area of two-month-old wild-type C57BL/6 mice. Eight months later, we observed the strong expression of GFP-tagged proteins, in all mice (Fig. 1a, Supplementary Fig. 1a) but only found substantial amyloid plaques in the hippocampus in mice that had been injected with vectors expressing APP (586–695) (Fig. 1b). Brains of mice injected with viral vectors encoding APP FL contained much less amyloid deposit, and amyloid plaques were virtually devoid with vectors encoding APP(1–373) or APP(1–585) (Fig. 1b). In the Morris Water Maze behavioral test, the latency to finding the submerged platform by mice infected with APP FL, APP(1–373) and APP(586–695) was significantly higher than the GFP control mouse groups (Fig. 1c). In the probe trail test, the percentage of time spent in the target quadrant was substantially less by these mice than GFP or APP(1–585) mice (Fig. 1d). However, swim speed remained similar among the groups (Supplementary Fig. 1b). Among these mice, the worst learning and memory activities were found in the APP(586–695) group. Based on electron microscopic analysis, synapse reduction in the hippocampal regions was tightly correlated with the cognitive behaviors; the most severe synaptic loss in CA1 area of the hippocampus was found in the APP(586–695) group (Fig. 1e, f). The LTP of fEPSPs in the hippocampal CA1 region, which is the molecular basis of learning and

memory, was diminished in APP(586–695) group compared with the control group (Fig. 1g). Paired-pulse ratios were smaller in APP(585–695) mice than in other groups, suggesting the presynaptic dysfunction (Fig. 1h). The I/O curve showed that the averaged slope of fEPSPs was smaller in mice expressing APP(586–695) than in control group (Fig. 1i). Taken together, these results (Fig. 1g–i) are all strong evidence that the most severe synaptic plasticity defects were found in mice injected with viral vectors encoding APP(586–695), and in alignment with the most prominent synaptic loss and the poorest learning and memory by this group. Thus, amyloid plaques deposited in the hippocampus of mice expressing APP(586–695) may diminish the synaptic plasticity and cognitive functions. Consistent with our previous findings [2], δ -secretase cleavage of APP at N585 accelerates amyloid plaque pathology by producing a much better substrate, APP(586–695), for BACE1.

δ -secretase-cleaved Tau(1–368) fragment initiates synaptic dysfunction and cognitive impairments

To assess whether the δ -secretase-cleaved Tau(1–368) fragment is sufficient to initiate formation of NFTs, we injected adeno-associated viruses (AAVs) expressing either human Tau FL, Tau(1–368) fragment, or control AAVs into the hippocampal CA1 area of wild-type C57BL/6 mice. After 3 months, we found that both Tau FL and Tau(1–368) were strongly overexpressed in the hippocampus (Fig. 2a), but pronounced Tau phosphorylation was found only in mice injected with vectors encoding Tau(1–368) (Fig. 2b). Impaired learning capabilities were found in the Tau(1–368) mice compared with control and Tau FL mice using the Morris Water Maze behavioral test. Tau(1–368) mice spent significantly less time in the target quadrant than the other two groups. Notably, defective learning and memory was also found in the mice in the Tau FL group when compared with the control group (Fig. 2c, d). In the swim distance analysis, the Tau(1–368) mice swam the longest distance among the three groups, followed by Tau FL mice (Supplementary Fig. 1c), fitting with cognitive function patterns, even though comparable swim speed was observed in these mice (Supplementary Fig. 1d). Using electron microscopic analysis, we found fewer synapses in the hippocampus of Tau FL mice than the control group, and the lowest synaptic density among the three groups was found in the Tau(1–368) mice (Fig. 2e). We observed this same order in the electrophysiology assay of the LTP with Tau(1–368) the worst, followed by Tau FL (Fig. 2f–h). Together, these results strongly support that both TauFL and Tau(1–368) overexpression trigger greater tau hyperphosphorylation, impairments of synaptic plasticity and cognitive functions, and synaptic loss and cognitive dysfunction compared with control group. In all of these outcome measures, overexpression of Tau(1–368) produces more severe pathology than Tau FL. Thus, δ -secretase cleavage of Tau at N368 site facilitates Tau aggregation and NFT pathologies, exacerbating its neurotoxicity.

APP(586–695) and Tau(1–368) fragments additively display AD pathologies and cognitive impairments

To determine whether the mixture of APP and Tau fragments from δ -secretase cleavage affects amyloid plaques and NFT pathologies via their interplay, we co-injected combinations of viral vectors into the mice, including: (1) GFP control; (2) Tau FL + APP FL; (3) Tau FL + APP (586–695); (4) Tau(1–368) + APP(586–695); and (5) Tau (1–368) +

APP(1–373). Three months later, human Tau was successfully expressed, based on immunohistochemical (IHC) staining of the hippocampal CA1 area with antibody HT7, as were the GFP-tagged APP fragments (Supplementary Fig. 2a, top and 2nd panels). Massive deposition of A β and tau was observed in Tau(1–368) + APP(586–695) mice (Supplementary Fig. 2a, 3–5th panel). A smaller number of Thioflavin S-positive signals were also detectable in the Tau FL + APP(586–695) and Tau FL + APP FL groups as well. Because the GFP and Tau(1–368) + APP (1–373) groups were negative for thioflavin S staining, we suggest that co-expression of both Tau(1–368) and APP (586–695) might generate the most A β among the combinations. We conducted NeuN staining, to quantify the numbers neurons in hippocampus, and found a significant reduction in the Tau(1–368) + APP(586–695) group compared to the other groups (Supplementary Fig. 2a, bottom panel; Supplementary Fig. 2b), indicating that combined Tau(1–368) and APP(586–695) overexpression results in demonstrable neuronal loss. Using antibody AT8, we found much stronger NFT immunoreactivity in both groups overexpressing Tau(1–368) than those overexpressing Tau FL (Fig. 3a). Using antibody 4G8, we found the strongest amyloid deposit signal in the Tau(1–368) + APP(586–695) group, followed by Tau FL + APP(586–695) and Tau FL + APP FL groups; whereas there was no positive signals detected in Tau(1–368) + APP(1–373) group or control group (Fig. 3a). Using an A β ELISA assay, we found that mice in both the Tau(1–368) + APP(586–695) and Tau FL + APP(586–695) groups had significantly more A β 1–40 and A β 1–42 than mice in the Tau FL + APP FL group (Fig. 3b). Immunoblotting was used to verify the expression of human Tau FL and Tau(1–368) and GFP-tagged APP FL and truncates (Supplementary Fig. 3a top and 2nd panels). The expression of the synaptic markers synaptotagmin and spinophilin were reduced in all of the experimental groups, relative to the control group (Supplementary Fig. 3b, top and 2nd panels). BACE1 expression levels were the most prominently elevated in mice in the Tau(1–368) + APP (586–695) group (Supplementary Fig. 3b, 3rd panel), consistent with the prominent deposition of A β in this group.

In the Morris Water Maze behavioral test, the latency to the submerged platform in all of the experimental groups was significantly longer than that observed in the GFP group, indicating that Tau and APP combined overexpression reduce learning capabilities (Fig. 3c). Similar results were found in analysis of both the percentage of time spent in the target quadrant and cross probe times, supporting the conclusion that the mixture of APP and Tau overexpression significantly attenuates memory relative to the control group (Fig. 3d, Supplementary Fig. 3c, d). Using Golgi staining, we found fewer dendritic spines on hippocampal pyramidal cells in all of the experimental groups than the control group (Fig. 3e). Using electrophysiology, we show that the capacity for synaptic plasticity was strongly diminished by the mixture of Tau and APP overexpression (Fig. 3f–h), consistent with the cognitive dysfunctions in these groups. Using ELISA to measure IL-1 α and TNF- α protein expression, we found that these markers of neuroinflammation were significantly elevated in all of the experimental groups than control mice (Fig. 3i, j). Remarkably, BACE1 mRNA levels were augmented in the Tau FL + APP(586–695) group and Tau(1–368) + APP (586–695) group (Fig. 3k), consistent with our BACE1 immunoblotting data. Thus, the combination of Tau(1–368) and APP(586–695) fragments robustly escalates the expression of BACE1. To further confirm that δ -secretase mediates the fragmentation of APP and Tau

in vivo, we expressed δ -secretase gene in the mouse brain and found that overexpression of δ -secretase induced the generation of APP(586–695) and Tau(1–368) fragments (Fig. 4a). Overexpression of δ -secretase in 3×Tg mouse model also induced the fragmentation of APP and Tau, phosphorylation of STAT1, overexpression of BACE1, leading to increased deposition of A β and Tau (Fig. 4b–e). We showed that compared to full-length Tau and APP, when the Tau(1–368) and APP(586–695) fragments are present in mice brain, they induce more robust amyloid plaques, NFTs, and neuroinflammation, all associated with extensive neuronal loss.

Tau(1–368) binds and enhances p-STAT1 transcriptive activity on BACE1

To search for the potential molecular mechanisms of how Tau(1–368) might stimulate BACE1 upregulation, we conducted a co-IP with the brain samples injected with control, Tau FL or Tau(1–368) AAVs using anti-human tau antibody HT7. The immunoprecipitated samples were analyzed by proteomic analysis. Since Tau(1–368) potentially escalates BACE1 mRNA and protein levels, we hypothesized that BACE1 transcription factors might be activated by Tau(1–368). Among numerous binding partners from Tau(1–368) samples, we found that STAT1, a well-characterized BACE1 transcription factor [31, 32], was present in immunoprecipitates from Tau(1–368) mice (Supplementary Fig. 4a). This observation was verified with immunoblotting (Supplementary Fig. 4b). Accordingly, in HEK293 cells stably transfected with APP (HEK293-APP cells), overexpression of GFP-STAT1 strongly elevated BACE1 levels, relative to GFP controls. GST-Tau(1–368) further enhanced the expression of BACE1 (Supplementary Fig. 4c, top-4th panels). Using the GST-pull down assay, we showed that Tau(1–368) has a much stronger affinity to STAT1 and p-STAT1 than Tau FL (Supplementary Fig. 4c, 5th and bottom panels). To explore whether Tau(1–368) selectively binds STAT family members, we employed STAT5a and STAT5b as comparison, and found that Tau (1–368) specifically interacted with STAT1 but neither of the STAT5s (Supplementary Fig. 4d). Using a truncation approach, we found that the Tau C-terminal (a.a. 369–441) fragment is dispensable for the binding activity, and the repeated domain (a.a. 256–368) is required for Tau to associate with STAT1 (Supplementary Fig. 4e). Since STAT1 is predominantly activated by tyrosine phosphorylation on its conserved residue Y701, we investigated whether this event plays any role in mediating Tau(1–368) and STAT1 complex formation. As expected, the unphosphorylatable mutant of STAT1 Y701F failed to bind Tau (1–368) (Supplementary Fig. 4f), suggesting that the upstream tyrosine kinases that phosphorylate STAT1 may regulate the association between STAT1 and Tau (1–368).

Since Tau(1–368) escalates BACE1 levels much more strongly in the presence of APP(586–695) than its absence, we wondered whether APP plays any role in this event. Consequently, we performed the experiment in HEK293 cells and HEK293-APP cells. Clearly, BACE1 protein induced by Tau(1–368) is much more abundant in the presence of APP (Supplementary Fig. 5a, top-3rd panels). Notably, p-STAT1 expression was stronger in HEK293-APP cells than HEK293 cells (Supplementary Fig. 5a, 4th & 5th panels). In previous studies, STAT1 is reported to be activated by both SGK1, also known as serum and glucocorticoid-regulated kinase 1, and JAK2 [34]. As expected, both kinases were activated in the presence of APP (Supplementary Fig. 5a, 6th-bottom panels). Using quantitative RT-

PCR analysis, we found that Tau(1–368) expression significantly increased BACE1 mRNA transcription in HEK293 cells, as compared with control or Tau FL groups. BACE1 mRNA levels were substantially higher in the presence of APP, with Tau(1–368) group producing the largest increase (Supplementary Fig. 5b). Using a BACE1 promoter luciferase assay, similar difference in activities were noted (Supplementary Fig. 5c). To assess whether APP(586–695) is sufficient to mediate the stimulatory activity of Tau(1–368), we co-transfected APP (586–695) with Tau FL or Tau(1–368) in HEK293 cells. Once again, we observed the greatest BACE1 expression levels in the Tau(1–368) group (Supplementary Fig. 5d). We used a luciferase assay to validate that BACE1 promoter activity was the highest in the presence of both Tau (1–368) and APP(586–695) (Supplementary Fig. 5e). Because APP(586–695) is a much better substrate for BACE1 than APP FL [2], we reasoned that the catalytic effect of APP(586–695) might be exerted by A β . Subsequently, we compared the stimulatory activity in the presence of different concentrations of A β in HEK293 cells, transfected with control, Tau FL, or Tau(1–368), respectively. In both immunoblotting and Luciferase activity assays, we found that A β augmented BACE1 protein and mRNA levels in a dose-dependent manner, with the maximal effect occurring in the Tau(1–368) group (Supplementary Fig. 5f,g). To test whether Tau(1–368) augmentation of BACE1 expression is due to enhanced activation of STAT1, we performed an electrophoretic mobility shift assay (EMSA). We found that the strongest promoter binding affinity was found with Tau(1–368), followed by Tau FL (Supplementary Fig. 5h). Using a chromatin immunoprecipitation (ChIP) assay, we found a similar activity pattern by Tau in facilitating STAT1 to bind BACE1 promoter (Supplementary Fig. 5i).

To examine whether BACE1 expression is regulated by Tau(1–368) with APP or A β in neurons, we prepared primary cortical cultures and infected neurons with AAV virus expressing control, Tau FL, or Tau(1–368) in the presence of APP FL, APP(586–695), or A β . As noted above, the strongest effect in promoting BACE1 expression in both APP FL and APP(586–695) groups was when the cells also expressed Tau(1–368). The same effect was observed in the presence of A β (Fig. 5a, top & 2nd panels). p-STAT1 and its upstream two kinases p-SGK1 and p-JAK2 were activated by APP FL and APP(586–695). The maximal activities were found in the presence of A β (Fig. 5a, 3rd-bottom panels). Using quantitative RT-PCR analysis, we found that BACE1 mRNA tightly coupled to its protein levels (Fig. 5b). To assess whether A β is required for the stimulatory effect of Tau(1–368) on BACE1 upregulation, we employed pharmacological inhibitors of α -, β -, γ -, and δ -secretase. Both β -secretase inhibitor and γ -secretase inhibitor repressed BACE1 expression and δ -secretase inhibitor also obviously reduced its protein levels, whereas treatments with α -secretase inhibitor had no significant effect (Fig. 5c), indicating that blockade of A β production completely abolishes the catalytic effect of Tau(1–368) on BACE1 upregulation. To investigate whether A β stimulates BACE1 expression via instigating Tau(1–368) to bind STAT1, we conducted a co-IP experiment in HEK293 cells, transfected with Tau FL or Tau(1–368) in the presence of GFP-STAT1. A dose-dependent effect of A β on BACE1 escalation was found. The strongest activity was found with Tau(1–368), followed by Tau FL. P-STAT1 signals were gradually augmented, as A β concentrations progressively increased (Fig. 5d, top-5th panels). The binding affinities by STAT1 toward Tau FL and Tau(1–368) were similar. Tau (1–368) strongly bound to p-STAT1, which was tightly

regulated by A β , whereas Tau FL weakly associated with p-STAT1 (Fig. 5d, 6th and bottom panels). Based on these observations, we suggest that Tau(1–368) may selectively bind active p-STAT1 and promote its transcriptive activity on BACE1. Using subcellular fractionation, we found that Tau(1–368) triggered more STAT1 nuclear translocation than Tau FL, where Tau(1–368) robustly interacted with STAT1 (Fig. 5e), further supporting our hypothesis. It has been shown previously that A β activates both SGK1 and JAK2 kinases [34, 35]. As a result, overexpression of SGK1 or JAK2 enhanced the stimulatory effect of Tau(1–368) on BACE1 expression. JAK2 overexpression produced a slight upregulation of BACE1 even in the absence of Tau(1–368) (Fig. 5f, top panel). Consequently, p-STAT1 was enhanced by both kinases (Fig. 5f, 2nd-bottom panels). On the other hand, inhibition of either kinase, by a pharmacological inhibitor, attenuated the effect of Tau(1–368), and the mixture of both inhibitors completely eradicated BACE1 expression. We made the same observation with the dominant-negative STAT1 Y701F, indicating that SGK1 or JAK2-activated STAT1 is accountable for the catalytic activity of Tau(1–368) on BACE1 upregulation (Fig. 5g, Supplementary Fig. 6a–e). To confirm that δ -secretase is indispensable for the promoting effect of Tau(1–368) on BACE1 upregulation, we prepared primary cultures from LGMN^{+/+} and ^{–/–} mice. We found that A β elicited robust BACE1 upregulation in wild-type neurons, but it lost the effects in LGMN^{–/–} neurons (Supplementary Fig. 5j). The activation of STAT1 was not affected, when δ -secretase was deleted. Expression of Tau(1–368) fragment in LGMN^{–/–} neurons restored the effect of A β treatment on BACE1 expression (Supplementary Fig. 5k). Remarkably, exogenous human A β treatment promoted the production of mouse A β in primary neurons, suggesting that A β initiates a chain reaction to further accelerate the generation of A β (Supplementary Fig. 5l). Hence, A β -triggered δ -secretase plays a critical role in escalating BACE1 levels via proteolytic fragmentation of Tau at the N368 site.

Knockout of STAT1 diminishes the stimulatory effect of Tau(1–368) on BACE1 expression

We show that Tau(1–368) resulting from δ -secretase cleavage binds p-STAT1 and promotes its nuclear translocation, escalating BACE1 levels in the presence of the δ -secretase-truncated APP(586–695) fragment or A β . To test whether STAT1 is indeed required for this Tau(1–368) stimulatory effect on A β in vivo, we employed STAT1 flox/flox mice. We injected control virus or virus encoding human Tau(1–368) and APP (586–695) into the hippocampus of mice with or without floxed STAT1 expression. Injection of AAVs encoding Cre recombinase successfully depleted STAT1 in the hippocampus of the homozygous floxed mice. The APP and Tau truncates were robustly expressed (Supplementary Fig. 7a). Using IHC analysis with anti-6E10, we found that amyloid plaques were highly expressed in control mice overexpressing Tau(1–368) and APP(586–695), and these were substantially inhibited, when STAT1 was depleted. Similar observations were made from tissue processed for Thioflavin S staining. We observed more AT8-positive neurons than HT7-positive neurons, suggesting some of the mouse tau were also phosphorylated (Supplementary Fig. 7a). Using immunoblotting we found that Tau(1–368) + APP(586–695) co-expression escalated BACE1 protein levels, which was abrogated when in the absence of STAT1 (Fig. 6a, Supplementary Fig. 6f). This effect was confirmed by quantitative RT-PCR for BACE1 mRNA (Fig. 6b). Synaptic protein expression was decreased by Tau(1–368) + APP(586–695) co-expression in the control group, relative to STAT1-null mice (Fig. 6a). Using ELISA

we found that (Tau(1–368) + APP(586–695))-induced A β was substantially reduced in the absence of STAT1 (Fig. 6c). In a Morris water maze study, depletion of STAT1 prevented the cognitive dysfunctions induced by co-expression of Tau(1–368) and APP (586–695) (Fig. 6d, e). In alignment with these cognitive function results, co-expression of Tau(1–368) and APP (586–695) significantly reduced the density of dendritic spines, which was reversed in the absence of STAT1 expression (Supplementary Fig. 7b). Using electrophysiological analysis, we found that knockout of STAT1 reversed the reduction in the capacity for synaptic plasticity that was induced by co-expression of the Tau(1–368) and APP(586–695) fragments (Fig. 6f–g). Neuro-inflammation triggered by Tau(1–368) + APP (586–695) co-expression also was diminished in STAT1-depleted mice (Supplementary Fig. 7c), as was the neuronal cell death induced by Tau(1–368) + APP (586–695) co-expression. Notably, fewer NeuN positive hippocampal neurons were found in control mice than STAT1-null mice (Supplementary Fig. 7d). STAT1 depletion also alleviated the activation of microglia in the Tau(1–368) + APP(586–695) co-expression mice (Supplementary Fig. 7a, 8th panel). However, STAT1 depletion did not affect δ -secretase-mediated cleavage of Tau and APP in cultured neurons (Supplementary Fig. 7e). We interpret these findings as strong support of the hypothesis that the additive effect Tau(1–368) and APP(586–695) fragments on amyloid plaques deposit is mediated by upregulating BACE1 via STAT1 in vivo.

Discussion

Most recently, we reported that δ -secretase is upregulated and activated during ageing. It cleaves both APP and Tau and mediates amyloid plaque and NFT pathologies in AD. Knockout of *LGMN* from 5XFAD or TauP301S mice diminishes the appearance of senile plaques or NFT in these animal models, also rescuing the cognitive defects. Moreover, we showed that δ -secretase is highly activated in human AD brains and CSF, and the fragmented APP and Tau truncates are found in human AD brains [2, 3]. The lysosome enzyme δ -secretase (AEP) can be secreted into the extracellular space and re-uptaken into the endosomes [36]. We found that δ -secretase co-localizes with APP on the cell surface, and also in the endosome and lysosome, where it cleaves APP and facilitates the production of A β . Also, δ -secretase has been reported to be leaked from the lysosome to the cytoplasm in human AD brains [37], where it cleaves tau. The results of these studies are interpreted as support for the hypothesis that δ -secretase is required for transgenic APP/PS1 or Tau P301S to exert the pathologic effects in AD onset and progression. However, it has remained unknown whether δ -secretase activation is sufficient to trigger AD pathogenesis. In the current study, we provide compelling in vitro and in vivo data from experiments designed to test this possibility. We show that overexpression of δ -secretase-shed APP(586–695) fragment but not other truncates (APP(1–373) or APP(1–585)) or APP FL is sufficient to initiate amyloid plaque deposit, resulting in synaptic impairment and cognitive dysfunctions. The APP (586–695) fragment is much more robust than APP FL in these events (Fig. 1). Overexpression of δ -secretase-cleaved Tau(1–368) but not Tau FL is adequate to trigger NFT formation, leading to pronounced synaptic loss and crippled learning and memory (Fig. 2). Overexpression of the combination of Tau(1–368) and APP(586–695), the products of δ -secretase digestion, results in the most remarkable AD pathological characteristic hallmarks, including amyloid deposition, NFTs, neuro-inflammation, and neuronal cell loss.

Furthermore, co-expression of Tau (1–368) and APP(586–695) induced A β deposition within 3 months, while APP(586–695) itself took 8 months to induce A β deposition (Fig. 3). Therefore, it seems clear that the Tau(1–368) and APP(586–695) truncates additively instigate AD pathogenesis. These observations suggest that δ -secretase-cleaved Tau(1–368) and APP(586–695) fragments may exacerbate each other's neurotoxic activities via their interplay, manifesting the full spectrum of the pathological features of AD.

In investigating the molecular mechanisms accounting for the additive effects of overexpression of Tau(1–368) and APP(586–695) truncates, we observed that BACE1 levels were much higher in the Tau(1–368) + APP(586–695) group than any other groups. It is worth noting that Tau(1–368) alone also strongly escalates BACE1 (Supplementary Fig. 5a–c), suggesting that Tau(1–368) somehow induces BACE1 upregulation. Employing co-IP and proteomic analysis, we found that STAT1, a well-characterized transcription factor for BACE1 [31, 32, 38, 39], selectively bound Tau(1–368) (Supplementary Fig. 4). Conceivably, cleavage of Tau by δ -secretase at N368 may relieve the steric hindrance of the N terminus and thereby enhance its binding affinity with STAT1. STAT factors are latent transcription factors in the cytoplasm that become activated by various extracellular stimuli, including cytokines and growth factors that bind to specific cell surface receptors. The activation of STAT proteins is largely mediated by phosphorylation of conserved tyrosine and serine residues in their C-terminal transactivation domain through Janus kinases (JAKs) and mitogen-activated protein kinases (MAPKs), which allows the activated STATs to dimerize and to translocate into the nucleus [40]. Notably, we find that Tau(1–368) selectively binds to p-STAT1 and promotes its nuclear translocation, leading to augmentation of BACE1 transcription (Fig. 5, Supplementary Fig. 4). Nonetheless, we found that the stimulatory effect of Tau(1–368) on BACE1 is dependent on the presence of APP or APP(586–695) (Supplementary Fig. 5). We reported recently that the δ -secretase-cleaved APP(586–695) fragment is a better substrate for BACE1 to generate A β than APP FL [2]. We also found a dose-dependent acceleratory effect on Tau(1–368)-triggered BACE1 transcription produced by A β (Supplementary Fig. 5). We interpret these observations to mean that A β might instigate the signaling cascades that dictate the stimulatory activity by Tau(1–368) on BACE1 escalation.

In previous studies A β was found to activate both JAK2 and SGK1 [34, 35]. A β increases SGK1 phosphorylation at S78, leading to phosphorylation of STAT1 Y701 and S727, and activation [34]. Accordingly, inhibition of these kinases diminishes p-STAT1 and represses BACE1 expression. Dominant-negative STAT1 Y701F abrogates activity of Tau(1–368), while overexpression of either JAK2 or SGK1 activates STAT1 and escalates BACE1 levels (Fig. 5). In alignment with the key role of STAT1 in mediating the promotion of BACE1 expression in vitro by Tau(1–368), knockout of STAT1 greatly abolishes the effects by Tau(1–368) and APP(586–695) co-expression, as most of the core AD pathological features triggered by co-expression of Tau (1–368) and APP(586–695) were inhibited (Fig. 6). Conceivably, A β -activated SGK1 or JAK2 phosphorylates STAT1, which strongly binds Tau(1–368) and translocates into the nucleus, where they bind the BACE1 promoter and upregulates its transcription and protein expression (Fig. 7).

Accumulating evidence supports the postulate that neuroinflammation plays a critical role in the development of AD. The activated glial cells excessively release pro-inflammatory mediators and cytokines that trigger neurodegenerative cascades [41, 42]. Inflammatory reactions and mediators augment APP expression and A β formation [31, 43] and transcriptionally upregulate mRNA and protein levels and enzymatic activity of BACE1 [44, 45]. For instance, IFN γ is a cytokine that is critical for innate and adaptive immunity. It has been reported that IFN γ -induced BACE1 expression is mediated by activation of JAK2 and ERK1/2 signaling pathways and direct binding of STAT1 to BACE1 promoter in astrocytes [31, 32]. Fitting with these findings, we also found that combined expression of Tau(1–368) and APP (586–695) elicited substantial neuroinflammation, and activation of both SGK1 and JAK2 (Fig. 3, Supplementary Fig. 3). Inhibition of both kinases completely blocked STAT1 activation and BACE1 upregulation (Fig. 5). In addition to BACE1, STAT1 is implicated in mediating the action of a number of inflammatory factors [46]. As a result, inhibition of JAK2 attenuates the increase in inflammatory markers in microglia from APP/PS1 mice [46].

The amyloid hypothesis states that the deposition of amyloid beta (A β) in plaques in the brain is the primary influence driving AD pathogenesis. A β proteolytically cleaved from APP initiates Tau hyperphosphorylation and aggregation and triggers neuronal cell death, leading to AD. This theory has dominated AD research for a long time. However, accumulating evidence challenges this causal role of A β in the degenerative cascade [47]. Here we show that δ -secretase-cleaved Tau(1–368) acts upstream of A β by stimulating active STAT1 nuclear translocation, leading to upregulation of BACE1, a rate-limiting enzyme for A β production. This striking discovery directly confronts the amyloid cascade hypothesis. In agreement with our results, it has been reported that deletion of tau from APP/PS1 transgenic mice decreases BACE1 expression and amyloidogenic processing of APP [48]. Furthermore, intraneuronal Tau modifications in human AD brain take place earlier than aggregated A β deposition in Braak-stage 1–3, and Tau tangles temporally develop either before or independent of A β plaques [26–28]. Using immunofluorescent co-staining with antibodies against Tau(1–368) and A β on human AD brains, Tau(1–368) cleavage occurred in Braak stage 1–2, before A β was detectable. As AD progressed, both Tau(1–368) and amyloid plaques were demonstrable in Braak stage 3–4; and senile plaques became more prominent in the later stage of 5–6 in both cortex and hippocampus (Supplementary Fig. 8a). δ -secretase-derived Tau(1–368) and APP (586–695) fragments were detected in the brain from Braak stage 3–6 patients (Supplementary Fig. 8b). Fitting with our observations that Tau(1–368) interacted with STAT1, we found that they co-localized in human AD brains as well (Supplementary Fig. 8c). Our innovative finding is consistent with the previous report that human secreted Tau increases amyloid-beta production in human primary cortical neurons. In vivo, neutralizing human Tau in Tau transgenic models also reduces A β levels [30]. Taken together, our data that δ -secretase-cut Tau(1–368) facilitates A β production introduces an additional layer of molecular regulation between the crosstalk of A β and Tau. Establishing a fresh connection between Tau and A β , our novel findings support a positive feed-forward regulation mechanism by which A β exacerbates the progression of AD driven by δ -secretase and Tau.

Supplementary Material

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Acknowledgements

This work was supported by grants from M.J. FOX Foundation (Grant ID 11137) and NIH RO1 (AG051538) to K. Y., and grant from National Natural Science Foundation (NSFC) of China (No. 81571249 and 81771382) to ZZ, and NSFC grant (No. 81528007) to KY and JZW. We thank ADRC at Emory University for human AD patients and healthy control samples. HEK293 cells stably transfected with APP were from Dr. Edward Koo, University of California.

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Fig. 1. δ -secretase-generated APP(586–695) fragment induces A β deposition, synaptic impairment, and cognitive defects. **a** Expression of GFP-tagged APP fragments in the hippocampus. Scale bar, 50 μm . **b** Thioflavin S staining showing the deposition of A β in the hippocampus of the virus-injected mice. Scale bar, 50 μm . **c**, Probe trail of Morris water maze test (mean $\pm$ SEM; $n = 10$ in each group). **d** Morris water maze analysis as latency traveled (s) and integrated latency (AUC) for GFP control, APP full-length, APP(1–373), APP(1–585), and APP(586–695) mice (mean $\pm$ SEM; $n = 10$ in each group). **e** Representative electron microscopy image of the synapses. Scale bar, 1 μm . **f** Synaptic density of mice hippocampus (mean $\pm$ SEM; $n = 6$ in each group). **g** LTP of fEPSPs (mean $\pm$ s.d.; $n = 6$ in each group). **h** The ratio of paired pulses (mean $\pm$ s.d.; $n = 6$ in each group). **i** I/O curves (relation between electrical stimulation and fEPSP slope) obtained in hippocampal slices prepared from mice injected with virus encoding GFP, APP full-length, APP(1–373), APP(1–585), and APP (586–695), respectively (mean $\pm$ s.d.; $n = 6$ in each group). All data were analyzed using one-way ANOVA. * $p < 0.05$ compared with GFP group

Fig. 2.

δ -secretase-generated Tau(1–368) fragment induces Tau phosphorylation, synaptic dysfunction, and cognitive impairments in mice. **a** Immunostaining with human tau specific antibody showing the expression of human tau in the hippocampus. Scale bar, 50 μ m. **b** Immunostaining with AT8 antibody showing the phosphorylation of tau in the hippocampus. Scale bar, 50 μ m. **c** Morris water maze analysis as latency traveled (s) and integrated latency (AUC) for GFP control, Tau full-length, and Tau(1–368) mice (mean $\pm$ SEM; $n = 10$ in GFP and Tau(1–368) group, $n = 9$ in Tau FL group). **d** Probe trail of Morris water maze test ($n = 10$ in GFP and Tau(1–368) group, $n = 9$ in Tau FL group). **e** Electron microscopy showing the synaptic density of mice hippocampus (mean $\pm$ SEM; $n = 6$ in each group). Scale bar, 1 μ m. **f** The ratio of paired pulses (mean $\pm$ s.d.; $n = 6$ in each group). **g** LTP of fEPSPs (mean $\pm$ s.d.; $n = 6$ in each group). **h** Averaged slope of fEPSPs from the I/O curves (mean $\pm$ s.d.; $n = 6$ in each group). All data were analyzed using one-way ANOVA. * $p < 0.05$ compared with the control group, # $p < 0.05$ compared with the Tau FL group

Fig. 3.

Co-expression of both APP(586–695) and Tau(1–368) fragments induce AD pathologies in mice. **a** Immunostaining with AT8 and 4G8 antibodies showing the phosphorylation of Tau and the deposition of A β in the hippocampus. Scale bar, 50 μ m. **b** Quantification of human A β in the brain lysate using ELISA (mean $\pm$ SEM, $n = 4$ in each group). **c** Morris water maze analysis as latency traveled (s) and integrated latency (AUC). **d** Probe trail of Morris water maze test ($n = 10$ in each group). **e** Golgi staining showing the dendritic spines from apical dendritic layer of the CA1 region. The spine densities are expressed as mean $\pm$ SEM ($n = 6$; $*p < 0.05$, one-way ANOVA). Scale bar, 10 μ m. **f** LTP of fEPSPs (mean $\pm$ s.d.; $n = 6$ in each group). **g** I/O curves obtained in hippocampal slices (mean $\pm$ SEM). **h** The ratio of paired pulses (mean $\pm$ SEM). **i, j** Quantification of IL1 α and TNF α in the brain lysate using ELISA (mean $\pm$ SEM, $n = 4$ in each group). **k** mRNA levels of BACE1 (mean $\pm$ SEM, $n = 4$ in each group). All data were analyzed using one-way ANOVA. $*p < 0.05$ compared with GFP group

Fig. 4.

Overexpression of δ -secretase promotes AD pathologies. **a** δ -secretase induced overexpression of BACE1 in wild-type mice. The mouse brains were injected with control virus or virus encoding δ -secretase. Western blot shows the expression of BACE1 and phosphorylation of STAT1 3 months after injection. **b** δ -secretase induced overexpression of BACE1 in 3×Tg mice. The 3×Tg mouse brains were injected with control virus or δ -secretase virus. Western blot shows the expression of BACE1 and phosphorylation of STAT1 3 months after injection. **c** Immunohistochemistry showing the deposition of A β and phosphorylation of Tau in 3×Tg mice injected with control virus or δ -secretase virus. **d** δ -secretase activity was increased in the brain of 3×Tg mice injected with δ -secretase virus. **e** A β ELISA showing the increased deposition of A β in the brain of 3×Tg mice injected with δ -secretase virus. Scale bar, 100 μ m. * p < 0.05 compared with the control group

Fig. 5. Tau(1–368) and A β activate p-STAT1 and promote the expression of BACE1. **a** Tau(1–368) and A β promote the expression of BACE1 and activates p-STAT1 in cultured neurons. Primary cultured neurons were infected with virus encoding APP FL, APP (586–695), Tau FL, and Tau 1–368 respectively. The expression of BACE1 and the phosphorylation of STAT1, JAK2, and SGK1 were analyzed using Western blot. **b** Quantitative RT-PCR showing the mRNA level of BACE1. **c** β -, γ -, δ -secretase inhibitors attenuate Tau(1–368)-induced BACE1 expression. **d** A β enhances the binding between Tau (1–368) and p-STAT1. HEK293 cells were co-transfected with GFP-STAT1 and Tau FL or Tau(1–368), and treated with A β fibrils. The binding between Tau(1–368) and p-STAT1 was analyzed using GST pull-down assay. **e** Subcellular fractionation assay showing Tau(1–368) promotes the nuclear translocation of p-STAT1. **f** Western blot showing overexpression of SGK1 and JAK2 activates STAT1 and enhances the effect of Tau(1–368) to promote BACE1 expression. **g**

Transfection of flag-tagged STAT1 Y701F and inhibitors of SGK1 and JAK2 blocks the Tau(1–368)-induced BACE1 expression. All data were analyzed using one-way ANOVA. * $p < 0.05$

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Fig. 6.

Depletion of STAT1 abolishes APP(586-695)/Tau(1-368) fragments-induced BACE1 upregulation. **a** Immunoblotting showing the effect of STAT1 depletion on the expression of BACE1 and synaptic markers regulated by APP(586-695)/Tau(1-368) co-expression. **b** RT-PCR showing that STAT1 depletion attenuate APP (586-695)/Tau(1-368) fragments-induced BACE1 expression (mean ± SEM; $n = 3$ in each group). **c** ELISA analysis of human Aβ. Depletion of STAT1 diminished Aβ upregulation elicited by co-expression of APP (586-695)/Tau(1-368) (mean ± SEM; $n = 3$ in each group). **d** Cross probe times analysis showing the effect of STAT1 depletion on the cognitive dysfunction induced by APP(586-695) and Tau(1-368) fragments (mean ± SEM; $n = 9$ in each group). **e** Latency to platform in Morris water maze test (mean ± SEM; $n = 9$ in each group). **f** Normalized fEPSP amplitude of the mice (mean ± SEM; $n = 10$ in each group). **g** LTP of fEPSPs (mean ± SEM; $n = 10$ in each group). All data were analyzed using one-way ANOVA. * $p < 0.05$

Fig. 7.

The schematic model of δ -secretase-generated APP and Tau fragments on BACE1 expression. δ -secretase cleaves APP, generating APP(586–695) fragment, which is further cleaved by β -secretase and γ -secretase to produce A β . A β induces the phosphorylation of SGK1 and JAK2, which subsequently activates STAT1. On the other hand, δ -secretase cleaves tau and generates Tau(1–368) fragment, which binds to activate STAT1, promoting its nuclear translocation. Activated STAT1 enhances the transcription of BACE1 and the production of A β .