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Upregulation of Alzheimer's amyloid- β protein precursor in astrocytes both in vitro and in vivo

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Abstract

The amyloid cascade hypothesis of Alzheimer's disease (AD) posits that amyloid- β (A β) protein accumulation underlies the pathogenesis of the disease by leading to formation of β -amyloid plaques, a pathologic hallmark of AD. A β is a proteolytic product of β -amyloid protein precursor (A β PP; APP), which is expressed in both neurons and astrocytes. Although considerable evidence shows that astrocytes may play critical roles in the pathogenesis of AD, the longitudinal changes of β -amyloid plaques in relationship to APP expression in astrocytes and cellular consequences are largely unknown. Here, we aimed to investigate astrocyte-related pathologic changes of β -amyloid and APP using immunohistochemistry and biochemical studies in both animal and cell models. We utilized 5XFAD transgenic mice and found age-dependent upregulation of APP in astrocytes demonstrated with astrocytic reactive properties, which followed appearance of amyloid plaques in the brain. We also observed that APP proteins presented well-defined punctate immunoreactivity in young animals that in contrast showed disrupted structures surrounding amyloid plaques in older mice. Moreover, we utilized astrocyte cell models and showed that pretreatment of A β ₄₂ resulted in down-stream astrocyte autonomous changes, including upregulation in APP and BACE1 levels, as well as prolonged amyloidogenesis that can be reduced by pharmacological inhibition of BACE1. Collectively, our results showed that age-dependent APP up-regulation in astrocytes is a key feature in AD, which will not only provide novel insights for understanding AD progression, but also may offer new therapeutic strategies for treating AD.

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Author contributions

Y.L., F.R., J.F.W., S.Z., H.S., S.Y.Z., S.J.M., and S.H.C. conducted the experiments. C.Z. conceived the experiments. R.E.T. and C.Z. interpreted the data. Y.L., F.R., and C.Z. prepared the manuscript. All authors approved the manuscript.

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Competing financial interests

None.

Keywords

Alzheimer's disease; amyloid- β ; amyloid pathology; amyloid- β protein precursor; astrocyte; autonomous; amyloidogenesis

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder, with a devastating prognosis that contributes to vast socioeconomic and humanitarian problems. In the United States, there are approximately 5.8 million people suffering from AD, and a worldwide estimation close to 24 million, with a prevalence rate exponentially rising after 65 years of age [1]. The risks of AD are associated with age and family inheritance, as well as a number of epigenetic and environmental factors. Specifically, AD is a genetically complex disorder and consists of two major forms: early-onset and late-onset AD based on the age of disease occurrence. The early-onset familial AD (FAD) cases (<60 years old; ~5% cases) are usually associated with mutations in the amyloid- β protein precursor (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) [2–4]. The late-onset AD cases (>60 years old; ~95% cases) are often related to a genetic variant ($\epsilon 4$) in *APOE*, which encodes apolipoprotein E4 protein [5, 6].

The hallmarks of AD in the brain are pathologically characterized by extracellular β -amyloid plaques primarily composed of amyloid- β (A β) peptides, and intracellular neurofibrillary tangles comprised of hyper-phosphorylated and aggregated tau proteins. The hippocampus, the brain area critical for learning and memory, is particularly affected in AD. Mounting evidence supports the “*A β hypothesis*” for the etiology of AD, stating that excessive accumulation of A β is the primary pathogenic event of AD. Accumulation of aggregated A β forms β -amyloid plaques, followed by neurofibrillary tangle formation which causes neuro-inflammation along with neurodegeneration, and ultimately results in dementia [2, 7–10]. A β is proteolytically generated via a serial cleavage of the type-I transmembrane amyloid- β protein precursor (APP) through β - and γ -secretases. APP expression is usually high in neurons, and relatively low in microglia and astrocytes in the brain, which all may undergo proteolytic cleavage to generate A β [11–14]. Specifically, APP is first cleaved by β -secretase to generate APP-carboxyl-terminal fragment- β (CTF β), which is subsequently cleaved by γ -secretase to produce A β peptides. Alternatively, APP cleavage by α -secretase generates APP-CTF α that will in turn prevent the generation of A β peptides. A β can be 37–43 amino acids long depending on the γ -secretase cleavage site. Most early-onset FAD mutations in *APP*, *PSEN1*, and *PSEN2* increase the levels of longer A β peptides, which drives the aggregation of A β into neurotoxic oligomeric assemblies [2, 3]. In late-onset AD, ApoE protein is highly expressed in the brain, primarily in astrocytes [15]. The AD risk associated isoform ApoE4 secreted by glia may increase A β levels by decreasing its clearance outside the brain and/or by stimulating neuronal A β production [16].

Although the amyloid cascade has been perceived as a compelling hypothesis explaining the etiology of AD, the astrocyte-related molecular phenotypes of β -amyloid deposition have not been fully elucidated. Emerging evidence shows that astrocytes, or the major glia cells,

play critical roles in amyloid pathology by various mechanisms [17–22]. Astrocytes may respond to β -amyloid plaques by changing their morphology to a reactive appearance [23] with the progression of AD [24]. Late stage AD-related astrocytes display an impaired ability in A β uptake and neuroprotection [25]. Interestingly, astrocytes expression of APP is upregulated following brain injury [11, 26, 27]. However, the longitudinal changes of β -amyloid plaques and APP expression in astrocytes related to AD pathology and cellular consequences remain unclear. Here, our studies were aimed at investigating the amyloid pathology of AD related to astrocytes using the well-studied 5XFAD mouse model and astrocyte cell models. The results of these studies can be useful in characterizing astrocyte-related Alzheimer's amyloid pathology, which should not only provide new insights toward a better understanding of AD, but also may offer potential therapeutic strategies in treating AD.

Materials and Methods

Animal and cell models:

All animal study procedures were approved by MGH IACUC and conformed to the animal welfare guidelines. The animals were housed with *ad libitum* access to food and water in a room with a 12-hr light and dark cycle in the animal facility. The 5XFAD mouse model has been previously reported that expresses APP^{Swedish/Florida/London} and PSEN1^{M146L/L286V} mutations [28, 29]. It recapitulates the major features of β -amyloid pathology including intraneuronal A β ₄₂ presentation and A β ₄₂-induced neurodegeneration. Differently aged female 5XFAD mice and age-matched littermate non-transgenic (non-tg) control mice were investigated. They were transcardially perfused under isoflurane anesthesia. After brain dissection, the tissue was processed for immunohistochemistry (IHC) analysis.

For cell culture, a previously described astrocyte cell model [30, 31] and the mouse primary astrocyte culture were utilized. Briefly, the E3 astrocytes were cultured in Advanced DMEM containing 10% FBS, 1% Glutamax, and 0.2% geneticin, and incubated in a humidified atmosphere (5% CO₂) at 37°C. The cells were exposed to 3 μ M A β ₄₂ for 24 hrs and washed with PBS 3 times to deplete the remaining medium. The cells were then cultured in fresh medium for an additional 24 or 48 hrs, before they were harvested and subjected to protein analyses.

For the mouse primary astrocyte culture, the astrocytes from the whole brains of embryonic day-18 mice were utilized and purchased from BrainBits® (Catalog #: C57EASTWB). They were grown following the protocol recommended by the manufacture. Briefly, the primary astrocytes were received and resuspended, and cultured with NbASTRO medium (Catalog #: NBAST) in PDL-coated culture plates and incubated in a humidified atmosphere (5% CO₂) at 37°C. The cells were used for the prolonged effects of A β ₄₂ on astrocyte amyloidogenesis. The cells were exposed to 3 μ M A β ₄₂ for 24 hrs and washed 3 times to deplete the remaining medium, and then replaced with fresh medium and cultured for an additional 24 hrs, before they were harvested and subjected to MSD A β analysis.

Chemicals and antibodies:

The A β ₄₂ peptide was from Anaspec (Catalog #: AS-24224) and the BACE1 inhibitor IV (labeled as BACE1i in context) was from EMD Millipore (Catalog #: 565788). Antibodies for Western blotting analysis included those targeting APP, BACE1 and β -actin; all of which were diluted 1:1000 from stocks. Specifically, the previously reported G12A antibody [32, 33] was a polyclonal antibody and used to detect full length APP and APP-CTFs. The antibody for BACE1, the β -secretase, was a monoclonal antibody from Cell Signaling (Catalog #: 5606S). The antibody for β -actin was from Cell Signaling (Catalog #: 3700s).

Immunohistochemistry (IHC):

The methods for IHC were previously reported [34, 35]. Animals were sacrificed, and the brains were dissected. The hemispheres used for IHC were first incubated in 4% paraformaldehyde at 4 °C for 28 hrs and then incubated in 30% sucrose solution in phosphate buffered saline (PBS) for cryoprotection as described previously [36]. Next, brains were snap frozen using Tissue-Tek® OCT™ compound (Sakura Finetek, Inc. USA) and sectioned sagittally on a sliding microtome (Leica SM 2010R). Once the tissue was processed, the free-floating brain sections were stained using IHC, focusing on the hippocampus and cerebral cortex. Afterwards, the brain sections were gently washed in Tris-buffered saline (TBS) and TBS containing 0.25% Triton X-100 (TBS+) 3 times for 10-min, followed by blocking for 2-h in TBS containing 0.25% Triton X-100 and 5% donkey serum (TBS++) (Sigma-Aldrich), followed with primary antibodies incubation in TBS+ overnight at 4 °C. The antibodies and dyes were used to detect amyloid plaques and APP proteins were shown (Table 1). Then, the brain sections were washed 3 times with TBS+ for 10 min and incubated with secondary antibodies at room temperature for 2-h. Hoechst 33342 was utilized to detect nuclei (Thermo Fisher Scientific) and 0.025% thioflavin-S (Sigma-Aldrich) was used to detect amyloid fibrils. Finally, the samples were washed 3 more times with TBS + and TBS respectively and mounted using PVA DABCO® mounting solution (Sigma-Aldrich). For immunofluorescence imaging was performed utilizing methods previously reported [9]. Briefly, the imaging brain sections spanning the hippocampus areas included different depths on the rostro-caudal axis. The photomicrographs were taken using a confocal immunofluorescence microscope (Olympus IX70) with 4x, 10x, or 40x objective lens. The exposure time for each specific immunostaining was maintained consistently between each slice and image analysis was performed using ImageJ (a public domain image analysis software). The quantifications of the IHC signal were based on reported methods [37], and focused on hippocampus. Briefly, we manually segmented the primary hippocampal proper CA areas (CA1–3) and the dentate gyrus (DG) region from images obtained using 4x magnifications. The intensity of amyloid plaques, APP, and APP/GFAP colocalization was defined as the accumulated lightness of all pixels (normalized to 0 to 255) in images that were higher than a predefined lightness threshold, then normalized by the number of pixels in the image. The lightness of APP-GFAP colocalization was defined as the product of the lightness of APP and GFAP and normalized to 0 to 255.

Protein extraction and Western blotting (WB) analysis:

The cell lysates were prepared using previously reported methods [38–41]. Briefly, cells were lysed in M-PER (Mammalian Protein Extraction Reagent, Thermo Fisher scientific) with 1X Halt protease inhibitor cocktail (Thermo Fisher Scientific). The lysates were collected, centrifuged at 10,000 g for 20-min, pellets were discarded, and supernatants were transferred to a new Eppendorf tube. Then, samples were analyzed for protein concentration and an equal amount of protein was applied to electrophoresis, separated on 4–12% gradient Bis-Tris SDS-PAGE gels (Novex, Life Technologies), and transferred to PVDF membranes (Novex, Life Technologies). Subsequently, the membranes were blocked for 30-min with SuperBlock™ (Thermo Fisher Scientific) and probed with primary antibodies, including G12A (1:2000), BACE1 (1:1000) and β -actin (1:1000), overnight at 4 °C. The membranes were then washed 5 times with TBS-T and incubated with appropriate secondary antibodies for 1-h at room temperature. After 5 additional washing steps, the Femto SuperSignal™ Substrate (Life Technologies) was used to develop the membranes. The membranes were developed by the Odyssey® Fc and analyzed by the Image Studio imaging software (LI-COR, Inc.).

A β analysis:

A β measurement was performed through the electrochemiluminescence-based multi-array MSD-A β MesoScale analysis, which has been previously reported [42–46]. In brief, 96-well plates were first blocked with Dilute 35 with shaking for 1 hr at room temperature, and the experimental samples and the MSD protein standards were then prepared in combination with detection antibodies, and incubated on a shaker, followed by washing and then adding the reading buffer. Subsequently, the electrochemi-luminescence signal was captured by the MesoScale SQ120 system. A β levels of the samples were analyzed based on protein standards.

Data analysis:

All the procedures assessing the non-transgenic control mice and transgenic mice were performed and compared side by side to decrease experimental errors. The results for protein analyses were presented as mean $\pm$ SEM. Statistical analyses were performed using Student's one-tailed t-test on animal-based IHC studies and two-tailed t-test on cell-based studies. Values of $p < 0.05$ were considered to be significant. Statistical analysis was performed using the PRISM GraphPad software.

Results

Age-dependent upregulation of astrocytic APP expression in AD transgenic animals

We first set out to investigate age-dependent changes in APP proteins in brains of 5XFAD and control animals. The previously characterized antibodies and dyes were utilized on brain sections by IHC to stain for APP and amyloid plaques. Brain sections were stained with G12A (red) and thioflavin-S (green), which is specific for identifying β -pleated sheets. As observed, thioflavin-S positive staining was present in the subiculum in 2-month old 5XFAD mice (Fig. 1A–B) but not in non-transgenic(non-tg) mice (Supplementary Fig. S1A–B),

which was in agreement with previously reported results [47]. Furthermore, there was close proximity, but not co-localization of APP and thioflavin-S stained molecules. Thus, β -sheets began to form with close proximity to APP proteins in 2-month old 5XFAD mice.

We then investigated APP expression in relationship to β -amyloid plaques in 10-month old 5XFAD and non-tg mice. Brain sections of these animals were stained with G12A (red) and thioflavin-S (green). Thioflavin-S stained signal was evident throughout the hippocampus and cortex in 5XFAD mice (Fig. 1C–D), but not non-tg mice (Supplementary Fig. S1C–D), as expected. We performed IHC quantification and focused on hippocampal CA1–3 areas and the dentate gyrus (DG) region and found that both amyloid plaques and APP signals of 10-month animals were respectively higher than those of 2-month old animals ($n=3$ in each group; $p<0.05$) (Fig. 1E–F). Interestingly, inspection of the high-power images indicated that the presence of APP immunoreactivity was disorganized and resided within close proximity to thioflavin-S stained plaques in 5XFAD mice. Furthermore, APP immunosignal demonstrated star-like structures that resembled the phenotype of astrocytes. Collectively, these results suggested a potential age-dependent upregulation of APP in astrocytes, which was associated with increased amyloid pathology of AD.

To elucidate age-dependent changes of APP in astrocytes, we used brain sections from 2- and 10-month old 5XFAD animals and stained by G12A (red) for APP and GFAP (green) for reactive astrocytes (Fig. 2). We found that APP and GFAP-responsive immunosignal could be identified in both groups of 5XFAD animals (Fig. 2A–B), but not in non-tg mice (Supplementary Fig. S2A–D). We also found that the dentate gyrus was one of the brain regions that displayed high signal for both G12A and GFAP signal, which were inspected through higher magnification (Fig. 2C–D). We showed that G12A immunosignal was greater in 10-month old mice than in 2-month old mice. There were evident co-localizations of APP and GFAP in 10-month, but not 2-month old 5XFAD animals (Fig. 2C–D). We performed IHC quantification and investigated whether hippocampal APP-GFAP colocalized signal in 10-month animals were significantly higher than that of 2-month old animals. Our results showed significant upregulation of APP-GFAP colocalization in 10-month old animals compared to 2-month old animals in the hippocampal CA1–3 areas (Fig. 2E) and the DG region (Fig. 2F), respectively ($n=3$ in each group; $p<0.05$). Thus, our studies confirmed an age-dependent increase of APP in astrocytes, which correlated with increased amyloid pathology. Our finding was consistent with previous findings of APP expression in Tg2576 animals [48] and also supported previous findings revealing regulatable and dynamic astrocytic APP expression [11]. Collectively, our findings suggest that formation of amyloid plaques precedes APP upregulation in astrocytes, which occurs age-dependently in association with amyloid pathology and may be used as a potential molecular phenotype for AD.

A β -induced upregulation of APP and BACE1 proteins, as well as prolonged amyloidogenesis in astrocytes

We further characterized cellular consequences of APP upregulation utilizing the previously reported E3 astrocyte model [30, 31] and mouse primary astrocytes. We found that E3 cells treated with A β ₄₂ for 24 – 48 hrs directly increased levels of full length APP (*data not*

shown), which agreed with previous results in cells [17] and recapitulated APP upregulation in AD transgenic animals [49, 50]. Because astrocyte has recently been shown to display critical autonomous functions [51], we next investigated whether A β may function as an upstream driver that leads to prolonged and downstream effects in increasing APP levels and amyloidogenesis through an autonomous function in astrocytes. Specifically, E3 cells were pretreated with 3 μ M A β ₄₂ peptide or control (DMSO) for 24 hrs, which were then washed and replaced with fresh medium, and incubated for additional 24 or 48 hrs. Although 3 μ M concentration may be higher than soluble A β levels in AD brains, it has been useful in cell models to represent the effects of A β accumulation and deposition in AD brains and similar range of concentrations has been reported [17][20]. We reasoned that an astrocyte autonomous function will be associated with APP upregulation and A β generation, even after A β withdrawal during the additional period of incubation. Indeed, WB analysis of cell lysates revealed upregulation of APP-FL levels at both 24 and 48 hrs comparing A β ₄₂ group to control; the increase did not reach statistical significance at 24 hrs while a statistically significance difference occurred at 48 hrs ($p < 0.05$) (Figs. 3A–B). We also found a significant upregulation of BACE1 levels at 48 hrs comparing A β ₄₂ group to control ($p < 0.05$) (Figs. 3A, C). Furthermore, we noticed a reduction of APP-FL levels comparing 48-hr control to 24-hr control, consistent with our previous report that higher cell density in relationship to reduced APP-FL levels [52].

Next, both E3 cells and mouse primary astrocytes were utilized and respectively treated with vehicle (DMSO), 3 μ M A β ₄₂ alone or in combination with the reported BACE1 inhibitor (BACE1i; 1000 nM) for 24 hrs, and then washed and replaced with fresh medium, and incubated for additional 24 hrs. MSD-A β analysis of medium showed that the A β ₄₂ peptide-pretreated cells displayed significantly higher A β ₄₂ levels, compared to control ($p < 0.05$) in both E3 astrocytes (Fig. 3D) and mouse primary astrocytes cell types (Fig. 3E), suggesting pretreat of A β leads to an autonomous function in astrocytes for prolonged amyloidogenesis. Cells pretreated with A β ₄₂ peptide in combination with BACE1i showed reduced medium A β ₄₂ levels compared to those treated with A β ₄₂ peptide alone (Fig. 3D–E); the differences revealed statistical significance in E3 cells ($p < 0.05$; Fig. 3D) while they did not reach statistical significance in mouse primary astrocytes (Fig. 3E). These results suggested that treatment of A β ₄₂ resulted in an astrocyte autonomous changes in association with increased amyloidogenesis, which can be reduced by pharmacological BACE1 inhibition. Collectively, our animal and cell-based studies showed that astrocytes displayed cell-autonomous mechanisms of A β generation, which closely correlates with increased amyloid pathology and APP upregulation in AD.

Discussion

This study was aimed at investigating age-dependent changes in APP in astrocytes that are related to amyloid pathology using both animal and cell models. We first performed analysis in 5XFAD mice of different ages, compared to age-matched littermate controls, and then investigated changes in astrocytic proteins and cellular effects of amyloidogenesis. Our results showed that amyloid pathology was related to an age-dependent upregulation of APP expression in reactive astrocytes. We also showed that A β accumulation in astrocytes utilized a feedforward autonomous mechanism, leading to prolonged amyloidogenesis

involving APP and BACE1 upregulation that could be reversed by pharmacological inhibition of BACE1.

Our findings provide novel insights on amyloid pathology and AD pathogenesis. Despite considerable study of amyloid pathology in AD, the longitudinal changes of amyloid pathology related to APP expression in astrocytes remain largely unknown. We first showed that there is an age-dependent up-regulation of APP expression in astrocytes in 5XFAD mice. We also demonstrated that there is increased APP immunoreactivity surrounding or in the vicinity of amyloid plaques, and that this is accompanied by disrupted structures indicating the occurrence of neurodegeneration. Interestingly, our results agree with previous studies showing increased expression of APP in astrocytes in other AD model animals [48] and models of brain injury and neuronal damage [11, 27]. While neurons and glia all express APP, APP proteins in neurons seemed to be more likely to be proteolytically cleaved with more A β generation than those in glia [12]. Furthermore, our cell-based studies suggested that the context of A β accumulation provided autonomous, or feedforward mechanisms in astrocytes for prolonged amyloidogenesis. It is important to note that an A β -induced equilibrium favoring a vicious cycle for amyloidogenesis can be inhibited by BACE1 inhibition, suggesting a therapeutic potential for AD. BACE1 and APP have complex biological roles and involve in AD as well as motor and cognitive deficits of brain injuries [53, 54]. However, the mechanisms of these areas have not been completely elucidated and require further investigation and careful evaluation. Our results warrant future studies to assess whether mechanisms other than BACE1, or AD-related genes and compounds, that reduce A β levels may affect astrocyte-related amyloidogenesis, e.g. γ -secretase modulators [46, 55], and curcumin and its analogs [41, 56], among others. These studies will be useful in evaluating the potential therapeutic effects of these compounds.

Our results are consistent with previous findings, showing the pathogenesis of AD in relationship to reactive astrocytes. Astrocytes and other nonneuronal cells, including microglia, and/or interneurons could generate A β peptides [12, 14] and play critical roles in regulating A β metabolism and plaque formation [22]. They may be directly influenced by existing β -amyloid plaques over the course of AD [23, 24] and may show phenotypic change, rather than a marked proliferation of glial precursors in AD brains [57]. Furthermore, the activity of astrocytes can be indirectly influenced by a perturbation in gut microbiota and lead to changes in the inflammatory cytokines and reduced deposition of amyloid plaques in the brain [58, 59]. Functionally, glia may affect neurobehavioral functions including activity-dependent plasticity and learning in mice [60]. The results of this study support the importance of investigating both cell-specific and synergistic functions of different cell types as well as their functions on neural circuitry in AD brains [61]. Our data suggested that increases of A β aggregation precede a reactive state of astrocytes that is related to increased expression in both GFAP and APP. In the future, it would be interesting to study whether or not changing the “deactivated” state of astrocytes may attenuate AD pathology and have a potential toward AD therapeutics.

This study has identified age-dependent changes in astrocytic APP, which should improve the overall understanding of APP changes in other pathophysiological conditions. One study showed that the endogenously low expression of APP in rat hippocampal astrocytes [27, 62]

was elevated by lesions in neighboring hippocampal neurons in a time-dependent, long-lasting manner [27]. These astrocytes may be the source of A β peptides that forms neuropathological amyloid plaques in AD. Additionally, ischemic brain injuries in rats lead to rapid induction of APP expression in reactive astrocytes [11]. These studies, together with this study, suggest that increased APP expression can be related to reactive astrocytes. Interestingly, APP is not necessarily related to reactive gliosis, which was supported by a study showing reactive gliosis in APP-deficient mice [63]. Besides APP-related functional changes in astrocytes, a recent study reported that microglial APP may regulate abilities to acquire a proinflammatory phenotype related to AD [64]. Collectively, the studies that are focused on elucidating the pathophysiological functions of APP in association with different cell types may have potentials to open up new research avenues and provide novel insight for a better understanding of AD.

In summary, our animal and cell-based results demonstrate that age-dependent APP up-regulation in astrocytes is a key phenotype in AD, which involves an A β -driven and autonomous function of astrocytes and is closely correlated with amyloid neuropathology. Our findings may not only provide novel insights for understanding the underlying mechanisms of AD progression, but also offer potential therapeutic strategies for treating AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Age-dependent upregulation of APP and amyloid plaques in brains of 5XFAD mice. Immunofluorescence (IF) microscopy-based IHC was utilized to investigate brain slices of 2-month (A-B) (n=3) and 10-month old (C-D) 5XFAD (n=3) mice, using the G12A antibody (red) for APP and thioflavin-S (green) for amyloid plaques, followed by IHC quantification (E-F). White box in A and C were further analyzed in higher magnification in B and D, respectively. The black/white images showed the separated staining signal and arrows denoted protein aggregation. Calibration bars: 100 μ m (A;C) and 10 μ m (B;D). Quantification of APP (E) and amyloid plaques (F) in the hippocampal CA1–3 areas and the DG region. Mean $\pm$ SEM; Student t-test; * p<0.05, ** p<0.01.

Figure 2.

Age-dependent changes of APP and GFAP proteins in brains of 5XFAD mice. IF was utilized to investigate brain slices of 2-month (**A-B**) ($n=3$) and 10-month old 5XFAD mice (**C-D**) ($n=3$), followed by IHC quantification (**E-F**). The brain slices were stained for GFAP by the GFAP-specific antibody (green), APP by G12A (red) and nuclei by Hoechst (blue). White box denoted regions in **A** and **C** that were further analyzed in higher magnification in **B** and **D**, respectively. The black and white images showed separated staining signal from APP and GFAP antibodies and Hoechst. Calibration bars: 100 μm (**A;C**) and 10 μm (**B;D**). Quantification of APP-GFAP colocalization intensity in hippocampal CA1–3 areas (**E**) and the DG region (**F**). Mean $\pm$ SEM; Student t-test; * $p<0.05$, ** $p<0.01$.

Figure 3.

Aβ₄₂-induced upregulation of APP and BACE1 levels and amyloidogenesis as an autonomous function of astrocytes. The E3 astrocytes were pretreated with 3μM Aβ₄₂ for 24 hrs, and then medium was washed to deplete the remaining Aβ₄₂ peptide and replaced with regular medium for additional incubation of 24 or 48 hrs; cell lysates were applied to quantitative WB for detection of APP and BACE1 (A-C). The E3 astrocytes (D) or mouse primary astrocytes (E) were treated with control (DMSO), 3 μM Aβ₄₂ alone or in combination with 1,000 nM BACE1 inhibitor (BACE1i) for 24 hrs, then washed and incubated with regular medium; medium was applied to MSD Aβ analysis (D-E). Mean ± SEM; n 3; * p<0.05, *** p<0.001 vs. corresponding controls; ## p<0.01, vs. Aβ₄₂; \$ p<0.05, comparing 48 hr Ctrl to 24 hr Ctrl; & p<0.05, comparing 48 hr Aβ₄₂ to 24 hr Aβ₄₂.

Table 1.

Overview of antibodies and dyes used in IHC of this study.

	Antibodies and Dyes	Host	Supplier	Dilution
G12A	APP and APP-CTFs	Rabbit	Custom made by Thermo Fisher Scientific	1:400
GFAP	Anti-GFAP	Mouse	Cell Signaling	1:400
Cy2, 3	Anti-Mouse	Donkey	Jackson ImmunoResearch	1:200
Cy2, 3	Anti-Rabbit	Donkey	Jackson ImmunoResearch	1:200
Amyloid fibrils	Thioflavin-S	Dye	Sigma-Aldrich	0.025%
Nuclei	Hoechst 33342	Dye	Thermo Fisher Scientific	1 µg/ml