Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

The Synaptic Vesicle Protein 2a Interacts With Key Pathogenic Factors in Alzheimer's Disease: Implications for Treatment

Yanyan Kong
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital https://orcid.org/0000-0001-7274-5683
Lin Huang
Shanghai University
Xuanting Liu
Shanghai University
Yinping Zhou
Shanghai University
Cuipin Liu
Shanghai University
Chencheng Zhang
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital
Bomin Sun
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital
Jiao Wang
Shanghai University
Yihui Guan (guanyihui@hotmail.com)
https://orcid.org/0000-0003-3001-7965

Research

Keywords: Alzheimer's disease, Neurodegeneration, Synaptic vesicle protein 2A, Tau, Aβ, PI3K signaling pathway

Posted Date: June 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32584/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background: Alzheimer's Disease(AD), a serious neurodegenerative disease,is pathologicallycharacterized by synaptic loss and dysfunction.Synaptic vesicle protein 2A (SV2A) is an indispensable vesicular protein specifically expressed in synapses and can be used as a biomarker for synaptic density. Nevertheless, the involvement of SV2A in the pathogenesis and development of ADand its relation to other hallmarks of AD pathology, such as amyloid precursor protein (APP), β-amyloid (Aβ), and tau protein are not fully understood.

Methods: We first examined and compared the mRNA levels of SV2A in the hippocampus of AD patients and non-AD subject in the Allen Brain database,thenwe constructed SV2A knockout mouse model.Using PET imagingwe compared the expression of Aβ in SV2A knockout mice and WT mice, analyze the relationship between SV2A and AD related proteins by quantitative real-time polymerase chain reaction (PCR), western blotting and ELISA.

Results: Our results showed thatthe expression of SV2A was downregulated in the hippocampus of AD patients.In addition,SV2A colocalized with APPandwas downregulated at Aβ deposition. Moreover, we used APPswe293T cells lines to either silence or overexpress SV2A and found that SV2A deficiency leads to a simultaneous increase in Aβand Tau hyperphosphorylation,whileSV2A overexpression was associated with down-regulation of BACE1 and APOE. In addition, evidence gained in the study points PI3K signaling pathway as a possible mediator in SV2A regulation influencing the incidence and development of AD.

Conclusions: Our research demonstrated that SV2A is an important regulator of AD, close interplay between SV2A and AD related proteins demonstrated in our studyprovide novel diagnostic and therapeutic opportunities of AD. This study provides guidelines and information regarding the mechanism of SV2A influence in the regulation of AD and possible future research of neurological diseases.

1. Background

Alzheimer's disease (AD) is the most common progressive neurodegenerative disease associated with aging [1, 2]. Typical pathological features of AD include senile plaques and neurofibrillary tangles[3, 4]. The primary component of senile plaques is β-amyloid (Aβ), which is secreted as a enzymatic digestion product of amyloid β-protein precursor (APP) by hydrolase [5–8]. while neurofibrillary tangles are composed of hyperphosphorylated Tau protein [2, 9–12]. Aβ deposition leads to senile plaques and neurofibrillary tangles, which cause damage and loss of brain neurons, compromise neurological function, and ultimately result in AD dementia. [5, 13]. However, the exact regulatory mechanisms of AD are complex and remain to be fully elucidated. Understanding the pathogenic mechanisms of Aβ and Tau in the AD brain is particularly important for AD prevention and treatment.[14, 15].

Synaptic loss and synaptic dysfunction are well established major mechanisms of pathological basis of mild cognitive impairment (MCI) in early AD and the structural basis of AD dementia [16–19]. However, the specific molecular mechanisms leading to compromised synaptic function remains unclear. Synaptic vesicle glycoprotein 2A(SV2A), an essential vesicle membrane protein ubiquitously expressed in synapses, could serve as a suitable biomarker for synaptic density[16, 20–23]. SV2A is also involved in synaptic vesicle transport, exocytosis, neurotransmitter release and regulates gene and protein expression [24]. Clinical studies have shown that SV2A dysfunction is involved in the pathogenesis of AD[24–26]. For example, positron emission studies using the SV2A radiotracer ^{11}C-UCB-J and ^{18}F-UCB-H have shown that AD patients had significantly less SV2A binding in the hippocampus compared to non-AD subjects [16, 27]. Therefore, targeting SV2A may provide a novel strategy for early diagnosis and treatment of AD[20, 28].

In this study, we found that overexpressing and silencing SV2A induced changes in expression level of Aβ both *in vitro* and *in vivo*. our results indicate that upregulation of SV2A decreases the relative expression level of AD-related genes. We found that Aβ and Tau expressions were significantly increased in certain brain regions of SV2A-KO mice by PET imaging technique. Moreover, we found that SV2A regulation of the occurrence and development of AD appeared to be mediated by the phosphatidylinositol 3-kinase (PI3K) signaling pathway. Our research provides evidence that SV2A is an important regulator of AD and lays the foundation for further research on neurological diseases.

2. Materials And Methods

2.1. Positron Emission Tomography (PET)

PET experiments were performed using a Siemens Inveon PET/CT system (Siemens Medical Solutions, Knoxville, United States) and conducted by the Huashan Hospital affiliated with Fudan University of China. Mice were first anesthetized with isoflurane and then subjected to micro-PET dynamic imaging, 48 hours after intravenously injected with a ^{18}F-AV45 radiotracer for quantitative Aβ and PBB3 for quantitative Tau *in vivo*, [29]. All experiments were carried out in compliance with national laws for the conduct of animal experimentation and were approved by the Animal Ethics Committee of Shanghai University.

2.2. Database Analysis

The datasets used were from the Aging, Dementia, and Traumatic Brain Injury Study (https://aging.brain-map.org/) [30]. and included 304 RNA-Seq samples collected from the hippocampus of 108 elderly donors with AD. We compared the amount of SV2A expression according to their normalized Fragments Per Kilobase of transcript per Million fragments mapped (FPKM) values.

2.3. Immunohistochemical Staining

Frozen sectioning was used to slice brain samples from AD patients and non-AD patients. Sections (20 μm) were rinsed three times with PBS and then permeabilized with 0.1% Triton X-100 in PBS, blocked with 5%BSA in PBS at room temperature for 30 minutes, and incubated overnight at room temperature with both rabbit SV2A antibody (1:1,000; Abcam) and mouse APP antibody (1:1,000; Covance). Subsequently, sections were washed for three times in PBS, followed by a 2-hours incubation with Goat Anti-Rabbit IgG(594) (1:300, Abcam) and Goat Anti-Mouse IgG(488) (1:300, Abcam). Sections were washed again, three times with PBS. The cell nuclei were then counterstained with 1 mg/mL 4',6-diamidino-2-phenylindole (DAPI; Sigma). After the counterstain, the sections were washed with PBST. Fluorescence intensity was detected by using a Zeiss LSM710 fluorescence microscope[31].

2.4. Genotyping by PCR

Genomic DNA extracted from tail was amplified by PCR using primers for the SV2A gene (forward, 5'-GAGGCTGTCTACACTGAGGTCTACTG-3') and (reverse, 5' -TGCGAGGCCAGAGGCCACTTGTGTAGC-3'). The first cycle used 94 °C for 3 minutes, followed by 33 cycles at 94 °C for 30 seconds, 62 °C for 35 seconds and 72 °C for 20 seconds. To identify mice with the Targeted allele, the following primers were used: forward, 5'-GAGGCTGTCTACACTGAGGTCTACTG-3' and reverse, 5'-CATAGCTGTCCCTCTTCTCTTATGGAG-3'; to produce a 293 bp amplicon in SV2A mice and a 183 bp amplicon in WT mice.

2.5. Cell Culture

APPswe293T cell line was kindly provided by Prof. F. Huang (Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai, China). It is a stable cell line that can continuously express APP and secrete Aβ into the medium. Cells were maintained in Dulbecco's Modified Eagle Medium (Invitrogen, United States) containing 10% fetal bovine serum (Invitrogen, United States) and 1% penicillin/streptomycin (Invitrogen, United States), 0.1% G418 (Invitrogen, United States). The cells were cultured in a humidified incubator with 5% CO2 at 37 °C. When the cells reached 80–90% confluence, they were detached with 0.25% trypsin (Gibco, Canada), seeded onto appropriate plates with fresh medium, and incubated overnight.

2.6. Infection

APPswe293T cells were seeded onto 12-well plates at a density of 2 × 105 cells/well and cultured overnight at 37 °C in an atmosphere of 5% CO2. The following day, cells were infected with the SV2A overexpressing virus (Lenti-CMV-SV2a-RFP) overexpressing virus control (Lenti-EGFP-Puro), SV2A silencing virus (Lenti-CMV-SV2a-shRNA-Puro), or a silencing virus control (Lenti-shRNA-mCherry), according to the manufacturer's protocol.

2.7. Immunofluorescence

APPswe293T cells were cultured in 12-well plates and infected aspreviously described, bright-field images were acquired using a Nikon microscope. Cells were then rinsed with PBS, fixed in 4% paraformaldehyde in PBS at room temperature (RT) for 10 min, and permeabilized with 0.1% Triton X-100 in PBS for 10 min.

Subsequently, the cells were blocked in 2% bovine serum albumin in PBS at RT for 1 h, followed by overnight incubation with rabbit anti-BACE1 Polyclonal primary antibody (1:500, Abcam, United States) and mouse anti- APP monoclonal primary antibody (1:: 500, Cell Signaling Technology) at 4 °C. Cells were then washed 3 times with PBS and incubated with three antibodies: donkey anti-mouse IgG secondary antibody Alexa 488 (1:1000, Abcam, United States), donkey anti-rabbit IgG secondary antibody Alexa 594 (1:1000, Abcam, United States) and donkey anti-goat mouse secondary antibody Alexa 647 (1:1000, Abcam, United States). The incubation sustained at RT for 2 h. Then DAPI was used for following incubation at RT for 5 min. Finally, the cells were washed 3 times with PBS. Fluorescence intensity was detected using a Zeiss LSM710 fluorescence microscope.

2.8. Total RNA Extraction, cDNA Synthesis, and Real-Time Quantitative PCR (qPCR)

APPswe293T cells were cultured in 12-well plates and infected as described above. Total RNA was extracted using a total RNA extraction kit (Promega, United States); 48 h post-infection, according to the manufacturer's protocol. The concentration of RNA was determined by measuring the absorbance at 260 nm, and 2 μg RNA was used for cDNA synthesis using an RT master mix (TaKaRa, Japan). qPCR amplification was performed least three times using a mixture of SYBR Green qPCR super mix (YEASEN, China), cDNA samples, and designated primers (Table 1). The relative gene expression levels were calculated by comparing the CT value of the gene of interest with that of Gapdh, the internal control.

Table 1
List of primers used for qPCR.

Gene name	**Primer sequence (5'-3')**
GAPDH	Upstream: GGAGCGAGATCCCTCCAAAAT
	Downstream: GGCTGTTGTCATACTTCTCATGG
APOE	Upstream: GTTGCTGGTCACATTCCTGG
	Downstream: GCAGGTAATCCCAAAAGCGAC
APP	Upstream: CTGGAGGGTATGGGGTTCC
	Downstream: TTGTCTTGCAGGACGTAGGTC
TAU	Upstream: GCTGTGGCAGGAACGAGAA
	Downstream: AGTTTGAAGGCTTGATGTCACG
BACE1	Upstream: TCTGTCGGAGGGAGCATGAT
	Downstream: GCAAACGAAGGTTGGTGGT
SV2A	Upstream: GCACAACGACGGAAAGAACG
	Downstream: CATGCCTTTGTTGGAGTCGG

2.9. Western Blotting

The total protein in the APPswe293T cells was extracted using a cell lysis buffer (Beyotime, China), according to the manufacturer's protocol. The infected cells in 12-well plates and the protein was extracted from the APPswe293T cells. After treatment, cells were washed twice with ice-cold PBS and the total protein was extracted using cell lysis buffer (Beyotime, China), according to the manufacturer's protocol. Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin in PBS at RT for 1 h and then incubated with the following primary antibodies at 4 °C overnight: mouse anti-APP (1: 1,000,Cell Signaling Technology), rabbit anti-Tau (1:1,000, Abways, China), rabbit anti-BACE1(1:1,000, Abcam, United States), rabbit anti-APOE (1:1,000, ABclonal, China), mouse anti-PI3K (1: 4,000,Proteintech, China), rabbit anti-ERK (1: 1,000,Proteintech, China) and rabbit anti-SRK (1: 1,000,Proteintech, China). The following day, the membranes were incubated with a mouse anti-GAPDH (1:1000, Abcam, United States) at RT for 1 h, followed by an infrared dye 700-conjugated goat anti-mouse IgG (1:10000, Zemed, United States) and an infrared dye 800-conjugated goat anti-rabbit IgG (1:10000, Zemed, United States) at RT for another hour. Visualization and quantification were carried out using the LI-COR Odyssey scanner and associated software (LI-COR Biosciences). The relative protein expression level was normalized to the Gapdh value from the same lane, Data were obtained from four immunoblots.

2.10. PI3K inhibition experiment

The cultured APPswe293T cells were divided into four groups: control group, SV2A, shControl and shSV2A groups, and the corresponding four groups were treated with PI3K inhibitor LY294002 (50 μm). After 48 hours of infection, cells were treated or not with50 μm LY294002 for 4 h, protein was extracted, and the expression of PI3K signaling–related molecules was detected by western blotting.

2.11. Statistical Analysis

All data were analyzed using EXCEL software and were presented as mean ± SEM. The mRNA and protein expression levels of APPswe293T cells were analyzed using a t-test. Significance level was set to $p < 0.05$.

3. Results

3.1. SV2A is downregulated in the brain of AD patients and colocalized with APP.

In order to investigate the molecular mechanism with which SV2A may be involved in the nervous system, we first examined and compared the mRNA levels of SV2A in the hippocampus of 108 AD patients and non-AD subject in the Allen Brain database[30]. Statistical analysis shows that the mRNA levels of SV2A were significantly decreased in AD patients as compared with non-AD project (Fig. 1A). This result

corroborates *in vivo* findings of reduced SV2A in the hippocampus of AD and MCI patients from SV2A PET imaging studies, and This suggest that SV2A play an important role in the molecular mechanism of AD[16, 27]. *In vivo* results showed significant changes of SV2A in AD patients' brain compared with non-AD patients, and it was consistent with the location of Aβ deposition, suggesting that SV2A related to the pathological changes of AD (Fig. 1B), For further analysis, we produced a SV2A knockout mice model deletion of exons 3, which resulted in the functional loss of the *SV2A* gene (Fig. 1C). PCR genotyping was used to identify homozygous knockout mice from their wild-type (WT) and heterozygous littermates (Fig. 1D)

3.2. Abrogation of SV2A promotes Aβ production

Senile plaques, a by-product of APP hydrolysis, is a key component of the neurodegeneration pathogenesis observed in AD[32, 33]. To further explore the relationship of of SV2A and Aβ, APPswe293T cells were cultured *in vitro*. After infected with overexpressing virus of SV2A, RNA and protein were extracted from cells and analyzed by quantitative real-time polymerase chain reaction (PCR) and western blotting. Overexpression of SV2A decreased the RNA level of APP (Fig. 2A). Conversely, the expression of APP proteins was upregulated in the SV2A knockdown group compared to the control group (Fig. 2B), indicating that SV2A may regulate AD pathogenesis through AD-associated proteins. In addition, APP and SV2A are co-localized in APPswe293T cells (Figure. 2C).

By using ELISA, the expression of Aβ secreted in the medium was assessed, 48 hours post infection and found that SV2A had a negative effect on Aβ expression, Overexpression of SV2A decreased the expression level of Aβ, downregulation of SV2A upregulated the expression of Aβ(Figure. 2D). Using PET imaging, we also compared the expression of Aβ in SV2A knockout mice and WT mice, showing that Aβ was over-accumulated in the brains of SV2A knockout mice, which further confirmed previous results (Figure. 2E)

3.3. SV2A regulates the expression level of BACE1 and APOE

We investigated the β-site APP cleaving enzyme 1 (BACE1) and the apolipoprotein E genes (APOE), two other genes associated with AD, for to their relationship with SV2A. They were selected because BACE1 is the first enzyme involved in APP splicing[34, 35], and APOE is a well-known genetic risk factor for late onset AD (LOAD) [36, 37]. We found that cells infected with SV2A overexpressing virus showed a significant decrease in BACE1 expression as observed at the mRNA level (Figure. 3A). Western blotting analysis showed similar results (Figure. 3B). In addition, immunofluorescence observations of BACE1 and SV2A in APPswe293T cells revealed that they are co-localized (Figure, 3C). Consistent with the BACE1 results, cells after transfection of a SV2A overexpressing virus showed a lower level of APOE expression, though there was no significant difference between the samples was observed by Western blotting (Figure. 3D). Overall, these results indicated that SV2A impacts on Aβ production by interacting with the AD-associated genes ((Figs. 2 and 3).

3.4. SV2A deficiency promotes Tau hyperphosphorylation

Another typical pathological feature of AD is the formation of neurofibrillary tangles, composed of hyperphosphorylated Tau protein [38], Thus, we examined whether SV2A regulates the expression level of hyperphosphorylated Tau protein. by using APPswe293T cells infected with overexpressing and silencing virus of SV2A. Results revealed that total Tau protein expression was downregulated in SV2A overexpressing cells as observed at mRNA level, while total Tau protein expression was not significantly different in SV2A silencing cells (Fig. 4A). Western blotting results also revealed a down-regulation of the total Tau protein expression in the SV2A overexpressing cells, alongside an up-regulation of total Tau protein expression in SV2A silencing cells (Fig. 4B). The agarose gel electrophoresis result of the product was consistent with that of qPCR analysis results (Fig. S1). We then assessed the expression level of the hyperphosphorylated Tau protein mutated at serine 356 (P-Tau-S356) and the hyperphosphorylated Tau protein mutated at Tyrosine 322 (P-Tau-Y322) by Western blotting (Fig. 4C-E). Results demonstrated an upregulation of P-TAU-S356 expression level in SV2A silencing cells. Taken together, these results indicate that SV2A is involved in AD by regulating the expression of hyperphosphorylated Tau protein (Fig. 4).

3.5. SV2A regulated AD through the PI3K signaling pathway but not ERK or SRC.

We next assessed the dynamics of SV2A-related signaling pathways. Results revealed that comparing with the control group, the expression of PI3K was significantly down-regulated at the protein level after infecting SV2A overexpressing virus, and that was significantly up-regulated after infecting SV2A-silencing virus. In contrast, the ERK and SRC signaling pathways showed no significant changes. To further validate these observations, APPswe293T cells were treated with a PI3K-specific inhibitor, LY294002 [39], and showed that the expression of PI3K was down-regulated in all groups, We then compared the PI3K expression levels between SV2A overexpressing cells and SV2A silencing cells treated with LY294002, and found no significant difference. In light of the above results, it can be speculated that SV2A influences AD via the PI3K signaling pathway.

Discussion

In the present paper, we found SV2A to be colocalized with APP and downregulated in hippocampus of AD patients (Fig. 1), Moreover, we showed that abrogation of SV2A promotes Aβ production (Fig. 2), and upregulation of SV2A downregulates the AD risk factors BACE1 and APOE (Fig. 3). Further, SV2A deficiency promotes Tau hyperphosphorylation (Fig. 4). Finally, SV2A regulation of the pathogenesis and development of AD appears to be mediated by the PI3K signaling pathway (Figs. 5). This study provides guidelines and information regarding the mechanism of SV2A influence in the regulation of AD and possible future research of neurological diseases.

Indeed, synaptic loss is a prominent AD pathology and a major structurally related factor for AD cognitive impairment[16, 40].SV2A is a synaptic vesicle protein with 12 transmembrane domains ubiquitously expressed in the central nervous system and has brain specificity[41]. It has been shown that people with AD had significantly less SV2A binding in the hippocampus compared to cognitively normal participants according to SV2A PET using ^{11}C-UCB-J and ^{18}F-UCB-H [16, 27]. The hippocampus is a critical part of the brain for memory formation, and an important site of early damage associated with Alzheimer's disease [42]. Here, we found a significant decrease of SV2A expression at mRNA level in the hippocampus of AD patients comparing with non-AD subjects as per the Allen Brain database analysis (Fig. 1), indicating that changes in SV2A expression influence synaptic function in AD.

It has been shown that the expression of SV2A was down-regulated in the hippocampus of AD patients, suggesting that SV2A may serve as a key regulator to AD related proteins. such as Aβ and APP, two hallmarks of AD [43]. Our study detected an increase in the expression levels of APP in APPswe293T cells infected by SV2A silencing virus (Fig, 2A, B). The results of immunofluorescence analysis of APP and SV2A (Fig. 2C), ELISA statistics analysis of the Aβ (Fig. 2D), and the result of brain observation by PET in SV2A KO mice (Fig. 2E) also validated the increase of APP in SV2A deficiency situation. It has been well documented that APP is stabilized by the $GABA_B$ receptor, at the cell surface, which leads to a reduction in APP proteolysis to Aβ[44]. Meanwhile, it has also been demonstrated that SV2A deficiency impairs its interaction with synaptotagmin 1 causing a specific disruption of synaptic GABA release, which in turns downregulates $GABA_B$ receptor expression [45]. Therefore, we speculated that down-regulation of SV2A may impair the inhibition of APP proteolysis to Aβ via reducing the expression of the $GABA_B$ receptor, thus leading to up-regulation of APP and Aβ. Yet there is also evidence showing that in AD, the SV2A loss takes precedence in glutamatergic rather than GABAergic nerve terminals[46], and the specific regulatory mechanism of SV2A on APP remains to be elucidated.

A previous study had inferred that APP and Aβ may be regulated by SV2A. In the present study, we found that SV2A overexpression downregulates BACE1 and APOE at both the mRNA and protein expression levels (Fig. 3). The depletion of BACE1, which initiates Aβ production by cleaving the extracellular domain of APP [47], has been reported to restore part of the synaptic function, indicating that BACE1 may be necessary for optimal synaptic activity and cognition[48]. APOE is a major risk factor for Alzheimer's disease (AD)[49]. Studies have shown that the level of BACE1 in the brain may be affected by APOE before the onset of AD[50, 51]. APOE allele 4 has been associated with an increased risk of AD[52]. In addition, APOE regulates multiple brain pathways to varying degrees including lipid transport, synaptic integrity and plasticity, and cerebrovascular function [53]. It has been reported that SV2A knockout appears to negatively regulate dendritic spine density and synaptic plasticity [54]. SV2A regulates the release of action potential dependent neurotransmitters and SV2A dysfunction impairs the release of synaptic GABA and glutamate [20] [55]. Inhibition of BACE1 can promote the activities of various cell receptor proteins in presynaptic or postsynaptic glutamatergic and GABAergic synaptic membranes[56]. Therefore, we can speculate that SV2A can further influence AD by mediating BACE1 and APOE to regulate synaptic receptor. However, some studies have shown that SV2A knockout prevents ApoE4 from

promoting BACE1 processing of APP which plays role in WT cells by promoting the co-localization of BACE1 and APP in vivo[57] [58]

It has also been reported that SV2A density was inversely correlated with Tau phosphorylation[26]. Therefore, we investigated the relationship between SV2A and Tau, and found that SV2A overexpression in APPswe293T cells resulted in significant decreases in Tau mRNA and protein levels (Fig. 4A). Although p-Tau-S356 and p-Tau-Y322 did not change significantly with SV2A overexpression, a slight upward trend was found in p-Tau-S356 of shSVA2 (Fig. 4B, C). Meanwhile, SV2A knockout led to significantly increased levels of Tau protein phosphorylation (Fig. 4D). It is known that Tau cross-links synaptic vesicles, thereby slowing their mobilization and ultimately reducing synaptic transmission during intense stimulation [59]. Tau-induced deficits in synaptic vesicle mobility prevent their recruitment into release and thus lead lower neurotransmission [60]. Further, overexpression of Tau is found to potently inhibit axonal transport [61]. Since SV2A is involved in the regulation of synaptic vesicle transport, exocytosis, and neurotransmitter release [62], overexpression of SV2A may alleviate AD-related symptoms by reducing the content of tau and thereby inhibiting the impairment of synaptic vesicle information transmission.

SV2A is a synaptic vesicle membrane protein involved in the regulation of neurotransmitter release, and thus is essential for the activation of crucial signaling pathways [63]. In this study, we found that the involvement of SV2A in the pathogenesis and development of AD appears to be mediated by the PI3K signaling pathway, as upregulation of SV2A downregulated the expression of PI3K (Fig. 5A), and treatment with the PI3K inhibitor LY294002 blocked this effect (Fig. 5D). Decreased levels of PI3K subunits, as well as blunted AKT kinase phosphorylation, have been observed in the brain of AD patients [64]. Insulin-like growth factor-2 (IGF-2) was reported to attenuates memory decline and amyloid plaques plaque formation in AD mouse model by activating the PI3K/AKT/CREB signaling pathway [65]., and insulin has been observed to promote neuron growth and synapse formation through the PI3K signal pathway [64, 66]. Therefore, we speculate that SV2A may play a role in promoting insulin growth factor secretion. However, further studies are needed to clarify the link between SV2A, the PI3K signal pathway, and AD pathogenesis and progression.

Conclusions

Our research demonstrated that SV2A is an important regulator of AD, close interplay between SV2A and AD related proteins demonstrated in our study provide novel diagnostic and therapeutic opportunities of AD. This study provides guidelines and information regarding the mechanism of SV2A influence in the regulation of AD and possible future research of neurological diseases.

Abbreviations

AD: Alzheimer’s disease; Aβ: β-amyloid; SV2A: Synaptic vesicle glycoprotein 2A; APP: amyloid precursor protein; FPKM: Fragments Per Kilobase of transcript per Million fragments mapped;

PET: Positron Emission Tomography; PI3K: phosphatidylinositol 3-kinase; RT: Room Temperature; MCI: Mild Cognitive Impairment

Declarations

Acknowledgements

We gratefully acknowledge Professor Tifei Yuan for reviewing the manuscript for critical comments.

Authors' Contribution

Yanyan Kong, Jiao Wang designed the experiments. Yanyan Kong and Lin Huang conducted most of the experiments, with assistance from Xuanting Liu, Yinping Zhou, Cuipin Liu. Yanyan Kong, Lin Huang and Xuanting Liu collected data and contributed to the statistical analysis. Yanyan Kong, Jiao Wang analyzed the data and wrote the manuscript. Yanyan Kong, Chencheng Zhang, Jiao Wang, Bomin Sun and Yihui Guan obtained funding and revised the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (Project No. 81571345, 81701732), Shanghai Municipal Science and Technology Major Project (No.2018SHZDZX03)

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethical Approval

All animals were treated in accordance with the International Guidelines for Animal Research. The study design was approved by the Animal Ethics Committee of Fudan University.

Consent for publication

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' information

1 Department of Functional Neurosurgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, China, 200025. 2 Laboratory of Molecular Neural Biology, School of Life Sciences, Shanghai

University, 99 Shang Da Road, China, 200444. 3 PET Center, Huashan Hospital, Fudan University, Shanghai, China, 200235.

References

1. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015;14:388–405.
2. TurabNaqvi AA, Hasan GM, Hassan MI. Targeting tau hyperphosphorylation via kinase inhibition-Strategy to address Alzheimers' disease. Current topics in medicinal chemistry. 2020.
3. Ulrich JD, Finn MB, Wang Y, Shen A, Mahan TE, Jiang H, et al. Altered microglial response to Abeta plaques in APPPS1-21 mice heterozygous for TREM2. Molecular neurodegeneration. 2014;9:20.
4. Wang Y, Ulland TK, Ulrich JD, Song W, Tzaferis JA, Hole JT, et al. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. The Journal of experimental medicine. 2016;213:667–75.
5. Myllykangas L, Polvikoski T, Reunanen K, Wavrant-De Vrieze F, Ellis C, Hernandez D, et al. ApoE epsilon3-haplotype modulates Alzheimer beta-amyloid deposition in the brain. Am J Med Genet. 2002;114:288–91.
6. Magnoni S, Esparza TJ, Conte V, Carbonara M, Carrabba G, Holtzman DM, et al. Tau elevations in the brain extracellular space correlate with reduced amyloid-beta levels and predict adverse clinical outcomes after severe traumatic brain injury. Brain. 2012;135:1268–80.
7. Kummer MP, Schwarzenberger R, Sayah-Jeanne S, Dubernet M, Walczak R, Hum DW, et al. Pan-PPAR modulation effectively protects APP/PS1 mice from amyloid deposition and cognitive deficits. Mol Neurobiol. 2015;51:661–71.
8. Reed-Geaghan EG, Savage JC, Hise AG, Landreth GE. CD14 and toll-like receptors 2 and 4 are required for fibrillar A{beta}-stimulated microglial activation. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2009;29:11982–92.
9. Noble W, Hanger DP, Miller CC, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. Front Neurol. 2013;4:83.
10. Pooler AM, Noble W, Hanger DP. A role for tau at the synapse in Alzheimer's disease pathogenesis. Neuropharmacology. 2014;76(Pt A):1–8.
11. Rodriguez-Martin T, Cuchillo-Ibanez I, Noble W, Nyenya F, Anderton BH, Hanger DP. Tau phosphorylation affects its axonal transport and degradation. Neurobiol Aging. 2013;34:2146–57.
12. Frost B, Gotz J, Feany MB. Connecting the dots between tau dysfunction and neurodegeneration. Trends Cell Biol. 2015;25:46–53.
13. Silvestre FJ, Lauritano D, Carinci F, Silvestre-Rangil J, Martinez-Herrera M, Del Olmo A, Neuroinflammation. Alzheimers disease and periodontal disease: is there an association between the two processes? J Biol Regul Homeost Agents. 2017;31:189–96.

14. Buckley K, Kelly RB. Identification of a transmembrane glycoprotein specific for secretory vesicles of neural and endocrine cells. J Cell Biol. 1985;100:1284–94.
15. Ma M, Dorstyn D, Ward L, Prentice S. Alzheimers' disease and caregiving: a meta-analytic review comparing the mental health of primary carers to controls. Aging Ment Health. 2018;22:1395–405.
16. Chen MK, Mecca AP, Naganawa M, Finnema SJ, Toyonaga T, Lin SF, et al. Assessing Synaptic Density in Alzheimer Disease With Synaptic Vesicle Glycoprotein 2A Positron Emission Tomographic Imaging. JAMA Neurol. 2018;75:1215–24.
17. Bello-Medina PC, Gonzalez-Franco DA, Vargas-Rodriguez I, Diaz-Cintra S. Oxidative stress, the immune response, synaptic plasticity, and cognition in transgenic models of Alzheimer disease. Neurologia. 2019.
18. Lauterborn JC, Cox CD, Chan SW, Vanderklish PW, Lynch G, Gall CM. Synaptic actin stabilization protein loss in Down syndrome and Alzheimer disease. Brain pathology. 2019.
19. Hatanpaa K, Isaacs KR, Shirao T, Brady DR, Rapoport SI. Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease. J Neuropathol Exp Neurol. 1999;58:637–43.
20. Tokudome K, Okumura T, Shimizu S, Mashimo T, Takizawa A, Serikawa T, et al. Synaptic vesicle glycoprotein 2A (SV2A) regulates kindling epileptogenesis via GABAergic neurotransmission. Sci Rep. 2016;6:27420.
21. Nicolas JM, Hannestad J, Holden D, Kervyn S, Nabulsi N, Tytgat D, et al. Brivaracetam, a selective high-affinity synaptic vesicle protein 2A (SV2A) ligand with preclinical evidence of high brain permeability and fast onset of action. Epilepsia. 2016;57:201–9.
22. Stockburger C, Miano D, Baeumlisberger M, Pallas T, Arrey TN, Karas M, et al. A Mitochondrial Role of SV2a Protein in Aging and Alzheimer's Disease: Studies with Levetiracetam. Journal of Alzheimer's disease: JAD. 2016;50:201–15.
23. Rokka J, Schlein E, Eriksson J. Improved synthesis of SV2A targeting radiotracer [(11)C]UCB-J. EJNMMI radiopharmacy and chemistry. 2019;4:30.
24. Mendoza-Torreblanca JG, Vanoye-Carlo A, Phillips-Farfán BV, Carmona-Aparicio L, Gómez-Lira G. Synaptic vesicle protein 2A: basic facts and role in synaptic function. 2013;38:3529–39.
25. Löscher W, Gillard M, Sands ZA, Kaminski RM, Klitgaard HJCD. Synaptic Vesicle Glycoprotein 2A Ligands in the Treatment of Epilepsy and Beyond. 2016;30:1055–77.
26. Metaxas A, Thygesen C, Briting SRR, Landau AM, Darvesh S, Finsen B. Increased Inflammation and Unchanged Density of Synaptic Vesicle Glycoprotein 2A (SV2A) in the Postmortem Frontal Cortex of Alzheimer's Disease Patients. Frontiers in cellular neuroscience. 2019;13:538.
27. Bastin C, Bahri MA, Meyer F, Manard M, Delhaye E, Plenevaux A, et al. In vivo imaging of synaptic loss in Alzheimer's disease with [18F]UCB-H positron emission tomography. Eur J Nucl Med Mol Imaging. 2020;47:390–402.
28. Onwordi EC, Halff EF, Whitehurst T, Mansur A, Cotel MC, Wells L, et al. Synaptic density marker SV2A is reduced in schizophrenia patients and unaffected by antipsychotics in rats. Nature

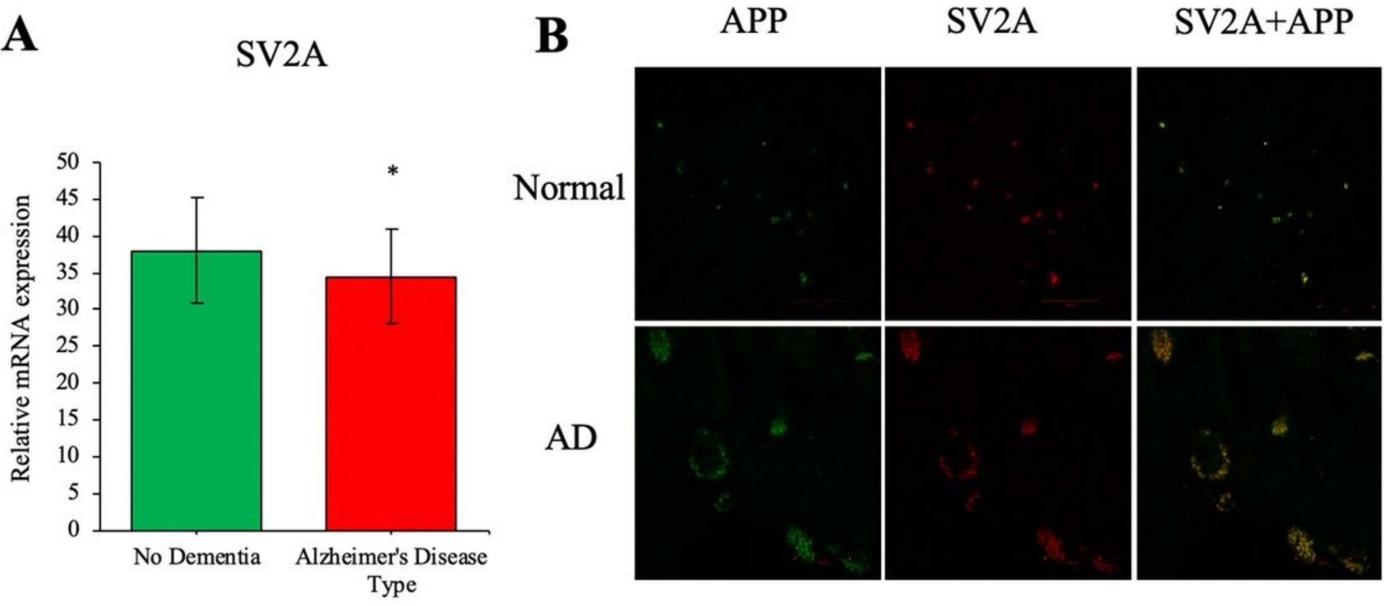
communications. 2020;11:246.

29. Ottoy J, Verhaeghe J, Niemantsverdriet E, De Roeck E, Wyffels L, Ceyssens S, et al. (18)F-FDG PET, the early phases and the delivery rate of (18)F-AV45 PET as proxies of cerebral blood flow in Alzheimer's disease: Validation against (15)O-H2O PET. Alzheimer's & dementia: the journal of the Alzheimer's Association. 2019;15:1172–82.
30. Miller JA, Guillozet-Bongaarts A, Gibbons LE, Postupna N, Renz A, Beller AE, et al. Neuropathological and transcriptomic characteristics of the aged brain. eLife. 2017;6.
31. Liu W, Lowenheim H, Santi PA, Glueckert R, Schrott-Fischer A, Rask-Andersen H. Expression of trans-membrane serine protease 3 (TMPRSS3) in the human organ of Corti. Cell Tissue Res. 2018;372:445–56.
32. Espeseth AS, Xu M, Huang Q, Coburn CA, Jones KL, Ferrer M, et al. Compounds that bind APP and inhibit Abeta processing in vitro suggest a novel approach to Alzheimer disease therapeutics. J Biol Chem. 2005;280:17792–7.
33. Mastromoro G, Gambardella S, Marchionni E, Campopiano R, Traversa A, Di Bonaventura C, et al. Unusual Segregation of APP Mutations in Monogenic Alzheimer Disease. Neuro-degener Dis. 2019;19:96–100.
34. Bettens K, Brouwers N, Engelborghs S, Van Miegroet H, De Deyn PP, Theuns J, et al. APP and BACE1 miRNA genetic variability has no major role in risk for Alzheimer disease. Hum Mutat. 2009;30:1207–13.
35. Ridler C. Alzheimer disease: BACE1 inhibitors block new Abeta plaque formation. Nature reviews Neurology. 2018;14:126.
36. Weintraub S, Teylan M, Rader B, Chan KCG, Bollenbeck M, Kukull WA, et al. APOE is a correlate of phenotypic heterogeneity in Alzheimer disease in a national cohort. Neurology. 2019.
37. Schreiber S, Schreiber F, Lockhart SN, Horng A, Bejanin A, Landau SM, et al. Alzheimer Disease Signature Neurodegeneration and APOE Genotype in Mild Cognitive Impairment With Suspected Non-Alzheimer Disease Pathophysiology. JAMA neurology. 2017;74:650–9.
38. Cho H, Choi JY, Lee HS, Lee JH, Ryu YH, Lee MS, et al. Progressive Tau Accumulation in Alzheimer Disease: 2-Year Follow-up Study. Journal of nuclear medicine: official publication. Society of Nuclear Medicine. 2019;60:1611–21.
39. Wang YQ, Lin Y, Zhao JD, Yang YT. [Inhibitory Effect of LY294002 on Proliferation of Multiple Myeloma Cells and Its Mechanism]. Zhongguo shi yan xue ye xue za zhi. 2017;25:1092–6.
40. Kelley BJ, Petersen RC. Alzheimer's disease and mild cognitive impairment. Neurologic clinics. 2007;25:577–609, v.
41. Zhang N, Gordon SL, Fritsch MJ, Esoof N, Campbell DG, Gourlay R, et al. Phosphorylation of synaptic vesicle protein 2A at Thr84 by casein kinase 1 family kinases controls the specific retrieval of synaptotagmin-1. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2015;35:2492–507.

42. Kramer JH, Schuff N, Reed BR, Mungas D, Du AT, Rosen HJ, et al. Hippocampal volume and retention in Alzheimer's disease. Journal of the International Neuropsychological Society: JINS. 2004;10:639–43.
43. Muller UC, Deller T, Korte M. Not just amyloid: physiological functions of the amyloid precursor protein family. Nature reviews Neuroscience. 2017;18:281–98.
44. Dinamarca MC, Raveh A, Schneider A, Fritzius T, Fruh S, Rem PD, et al. Complex formation of APP with GABAB receptors links axonal trafficking to amyloidogenic processing. Nature communications. 2019;10:1331.
45. Ohno Y, Tokudome K. Therapeutic Role of Synaptic Vesicle Glycoprotein 2A (SV2A) in Modulating Epileptogenesis. CNS & neurological disorders drug targets. 2017;16:463–71.
46. Govindpani K, Calvo-Flores Guzman B, Vinnakota C, Waldvogel HJ, Faull RL, Kwakowsky A. Towards a Better Understanding of GABAergic Remodeling in Alzheimer's Disease. International journal of molecular sciences. 2017;18.
47. Yan R, Vassar R. Targeting the beta secretase BACE1 for Alzheimer's disease therapy. Lancet Neurol. 2014;13:319–29.
48. McConlogue L, Buttini M, Anderson JP, Brigham EF, Chen KS, Freedman SB, et al. Synaptic The Journal of biological chemistry. 2007;282:26326–34.
49. Prendecki M, Florczak-Wyspianska J, Kowalska M, Ilkowski J, Grzelak T, Bialas K, et al. APOE genetic variants and apoE, miR-107 and miR-650 levels in Alzheimer's disease. Folia neuropathologica. 2019;57:106–16.
50. Decourt B, Gonzales A, Beach TG, Malek-Ahmadi M, Walker A, Sue L, et al. BACE1 levels by APOE genotype in non-demented and Alzheimer's post-mortem brains. Curr Alzheimer Res. 2013;10:309–15.
51. Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nature reviews Neurology. 2013;9:106–18.
52. Dai J, Johnson ECB, Dammer EB, Duong DM, Gearing M, Lah JJ, et al. Effects of APOE Genotype on Brain Proteomic Network and Cell Type Changes in Alzheimer's Disease. Front Mol Neurosci. 2018;11:454.
53. Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. Nature reviews Neurology. 2019;15:501–18.
54. Cohen JE, Lee PR, Chen S, Li W, Fields RD. MicroRNA regulation of homeostatic synaptic plasticity. Proc Natl Acad Sci USA. 2011;108:11650–5.
55. Lepannetier S, Gualdani R, Tempesta S, Schakman O, Seghers F, Kreis A, et al. Activation of TRPC1 Channel by Metabotropic Glutamate Receptor mGluR5 Modulates Synaptic Plasticity and Spatial Working Memory. Frontiers in cellular neuroscience. 2018;12:318.
56. Yan R, Fan Q, Zhou J, Vassar R. Inhibiting BACE1 to reverse synaptic dysfunctions in Alzheimer's disease. Neurosci Biobehav Rev. 2016;65:326–40.

57. Swarup V, Geschwind DH. Alzheimer's disease: From big data to mechanism. Nature. 2013;500:34–5.
58. Rhinn H, Fujita R, Qiang L, Cheng R, Lee JH, Abeliovich A. Integrative genomics identifies APOE epsilon4 effectors in Alzheimer's disease. Nature. 2013;500:45–50.
59. Zhou L, McInnes J, Wierda K, Holt M, Herrmann AG, Jackson RJ, et al. Tau association with synaptic vesicles causes presynaptic dysfunction. Nature communications. 2017;8:15295.
60. McInnes J, Wierda K, Snellinx A, Bounti L, Wang YC, Stancu IC, et al. Synaptogyrin-3 Mediates Presynaptic Dysfunction Induced by Tau. Neuron. 2018;97:823–35 e8.
61. Chee F, Mudher A, Newman TA, Cuttle M, Lovestone S, Shepherd D. Overexpression of tau results in defective synaptic transmission in Drosophila neuromuscular junctions. Biochemical Society transactions. 2006;34:88–90.
62. Mendoza-Torreblanca JG, Vanoye-Carlo A, Phillips-Farfan BV, Carmona-Aparicio L, Gomez-Lira G. Synaptic vesicle protein 2A: basic facts and role in synaptic function. Eur J Neurosci. 2013;38:3529–39.
63. Madeo M, Kovacs AD, Pearce DA. The human synaptic vesicle protein, SV2A, functions as a galactose transporter in Saccharomyces cerevisiae. J Biol Chem. 2014;289:33066–71.
64. Gabbouj S, Ryhanen S, Marttinen M, Wittrahm R, Takalo M, Kemppainen S, et al. Altered Insulin Signaling in Alzheimer's Disease Brain - Special Emphasis on PI3K-Akt Pathway. Front NeuroSci. 2019;13:629.
65. Xia L, Zhu X, Zhao Y, Yang G, Zuo X, Xie P, et al. Genome-wide RNA sequencing analysis reveals that IGF-2 attenuates memory decline, oxidative stress and amyloid plaques in an Alzheimer's disease mouse model (AD) by activating the PI3K/AKT/CREB signaling pathway. International psychogeriatrics. 2019:1–13.
66. Liem M, Ang CS, Mathivanan S. Insulin Mediated Activation of PI3K/Akt Signalling Pathway Modifies the Proteomic Cargo of Extracellular Vesicles. Proteomics. 2017;17.

Figures



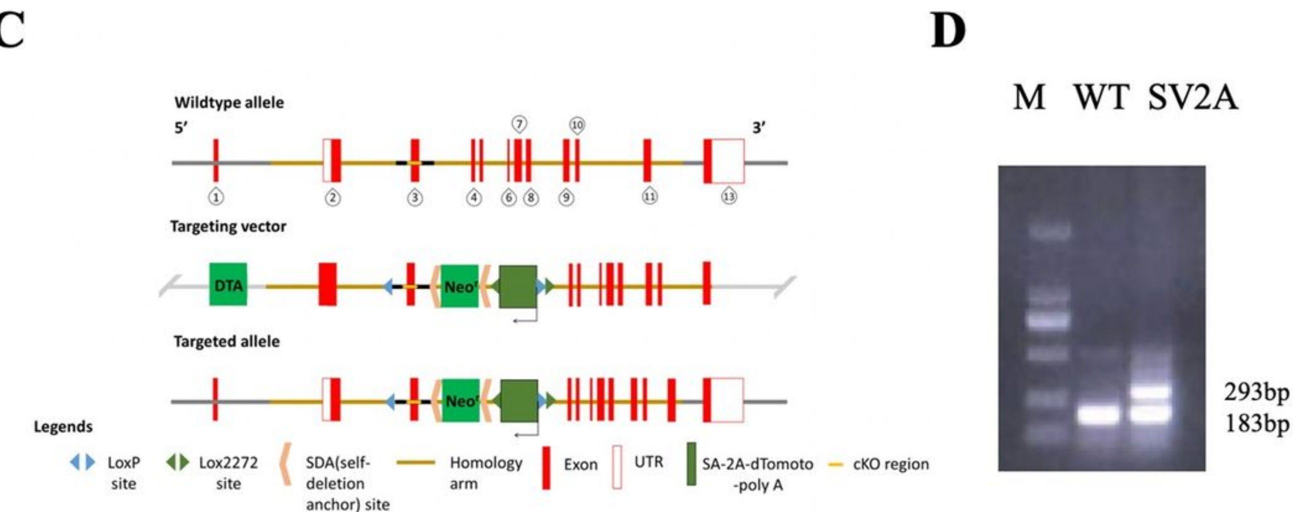


Figure 1

SV2A expression decreased and its pattern changed in AD patients.(A) Comparation of relative SV2A mRNA expression levels in the hippocampus of no dementia patients and AD patients. The expression level was normalized to the mean expression level of no dementia patients. Data comes from the Allen Brain database. For no dementia patients and AD patients�n. no dementia =30n. AD patients =30. (B) Immunofluorescence observation of APP (green) and SV2A protein (red) in the hippocampus of no dementia patients and AD patients reveals a change in the morphology of SV2A protein and a co-localizationof APP and SV2A protein in the hippocampus of AD patients. Scale bar, 20 μm. (C-D) The construction of a mouse SV2A conditional knockout model. (C) Scheme showing the targeting strategy for disruption of the SV2A gene. Exon 3 was selected as conditional knockout region.(D) Agarose gel

electrophoresis was used to identify the genotype of the SV2A mouse model. The lanes from left to right are the marker, DNA of wildtype mouse, DNA of SV2A+/- mouse. Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA). *p< 0.05.

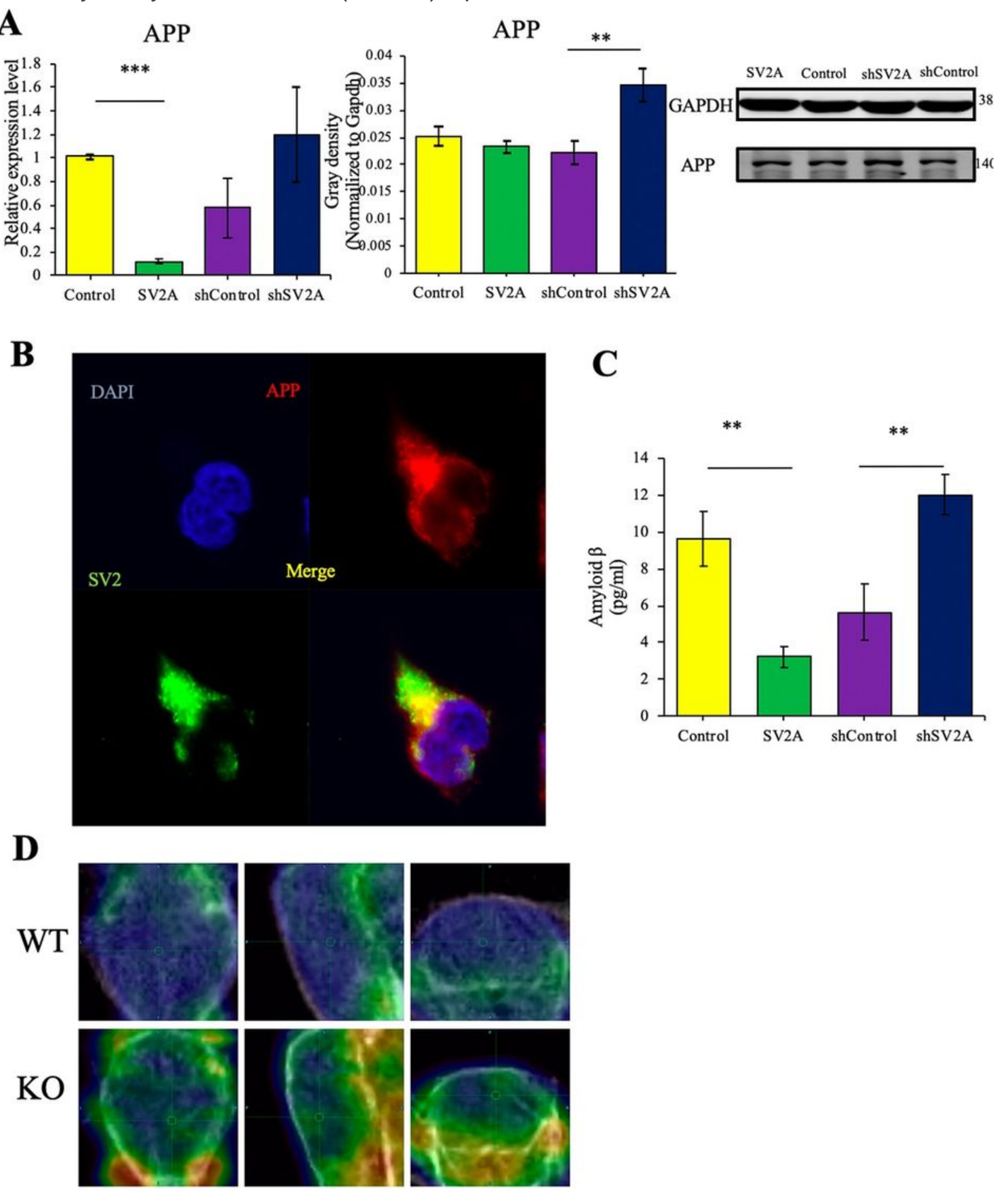


Figure 2

SV2A regulates the expression level of APP. (A) qPCR detection of APP mRNA expression level and western blotting detection of APP expression levels in APPswe293T cells from the following four groups:

Control, cells infected with SV2A overexpressing virus(SV2A), shControl, and cells infected by SV2A silencing virus(shSV2A). For qPCR, the expression level was normalized to the mean expression level of the Control group. For western blotting, the gray density was normalized to the mean gray density of GAPDH. (B) Immunofluorescence observation of the APP (red) and SV2A (green) in APPswe293T cells. The nuclei were counterstained with DAPI (blue). Scale bar, 40 μm. (C) Statistical analysis of ELISA detection of APPswe293T cells from the Control, SV2A, shControl and shSV2A groups. (D) Brain observation by PET in WT and SV2A KO mice. For each group of APPswe293T cells, n=3. Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA). **p< 0.01.***p < 0.001.

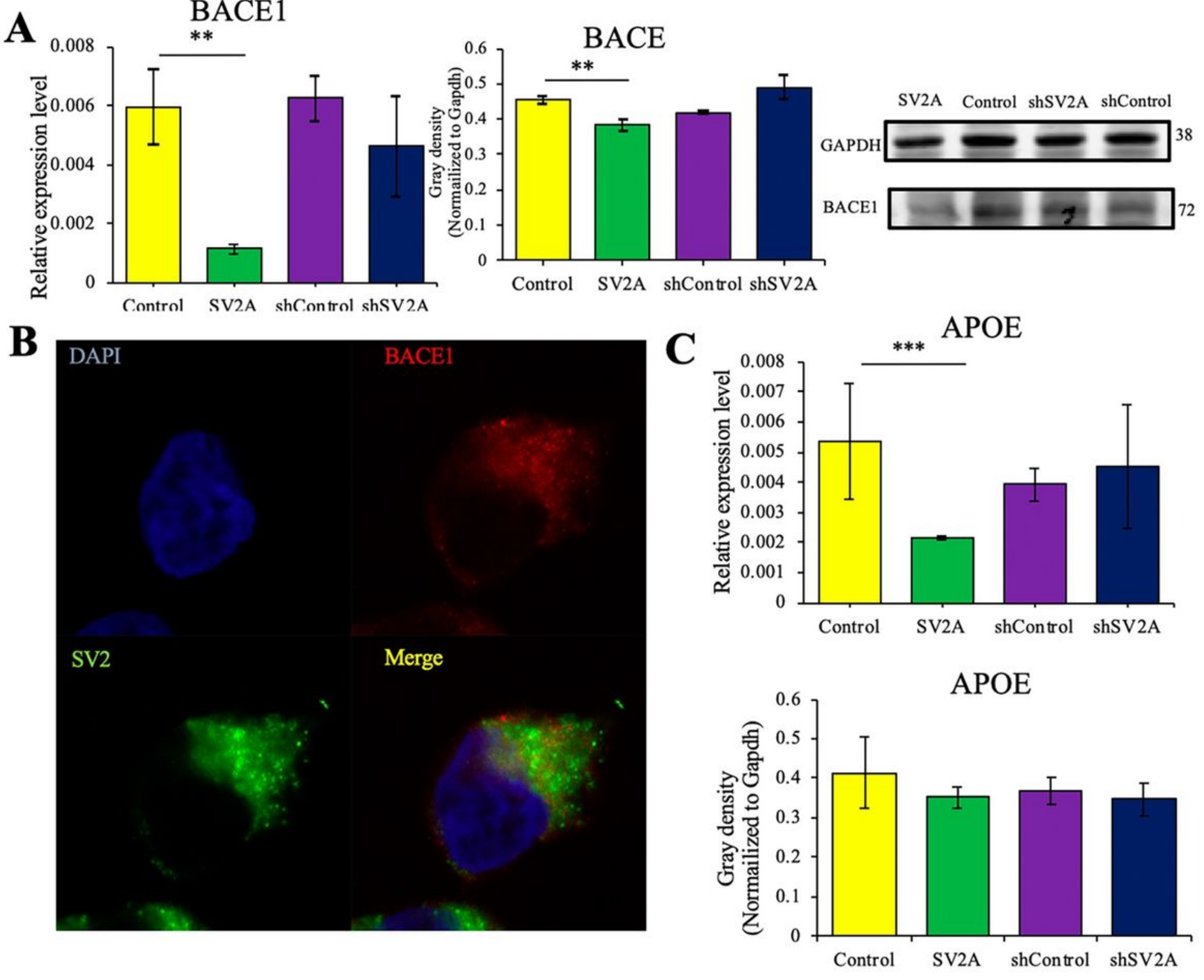


Figure 3

SV2A regulates the expression level of BACE1 and APOE. (A) qPCR detection of BACE1 mRNA expression level and western blotting detection of BACE1 expression levels in APPswe293T cells from Control, SV2A, shControl, and shSV2A groups. For qPCR, the expression level was normalized to the mean expression

level of theControl group. For western blotting, the gray density was normalized to the mean gray density of GAPDH. (B) Immunofluorescence observation of the BACE1 (red) and SV2A (green) in APPswe293T cells. The nuclei were counterstained with DAPI (blue). Scale bar, 40 μm. (C) qPCR detection of APOE mRNA expression level and western blotting detection of APOE expression levels in APPswe293T cells from Control, SV2A, shControl, and shSV2A groups.For qPCR, the expression level was normalized to the mean expression level of theControl group. For western blotting, the gray density was normalized to the mean gray density of GAPDH. For each group of APPswe293T cells, n=3. Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA). **p < 0.01.***p < 0.001.

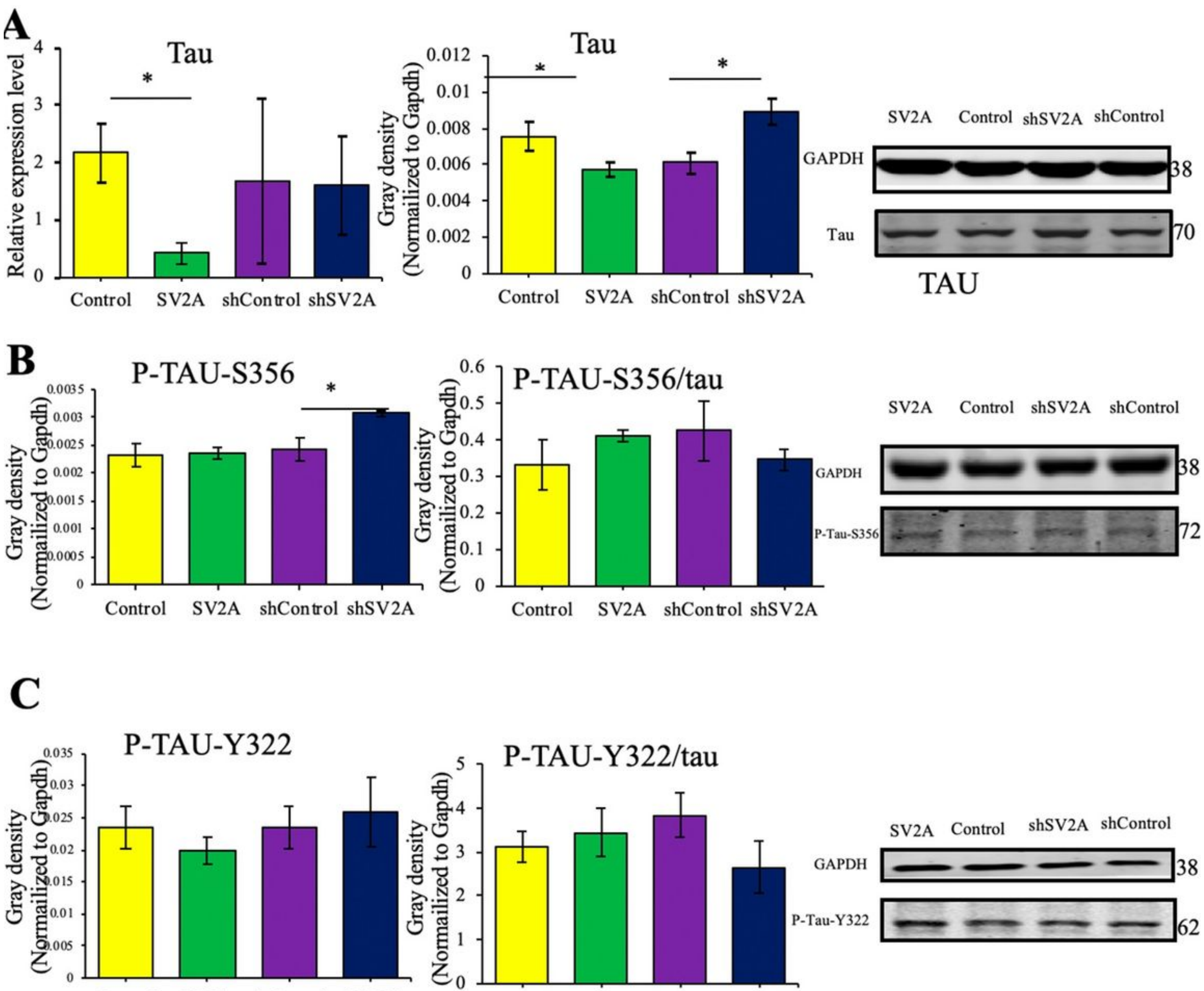


Figure 4

SV2A deficiency promotes Tau hyperphosphorylation. (A) qPCR detection of Tau mRNA expression level and western blotting detection of the Tau protein expression levels in APPswe293T cells from Control, SV2A, shControl, and shSV2A groups. For qPCR, the expression level was normalized to the mean

expression level of the Control group. For western blotting, the gray density was normalized to the mean gray density of GAPDH. (B) Western blotting detection of P-Tau-S356 protein (Phosphorylated tau protein mutated at serine 356)expression levels in APPswe293T cells from SV2A, Control, shSV2A, and shControl groups. The gray density was normalized to the mean gray density of GAPDH. (C) Ratio of p-Tau-S356 and Tau protein expression levels in APPswe293T cells from SV2A, Control, shSV2A, and shControl groups. The gray density was normalized to the mean gray density of GAPDH. (D) Western blotting detection of the P-Tau-Y322 protein (phosphorylated Tau protein mutated at Tyrosine 322) expression levels in APPswe293T cells from SV2A, Control, shSV2A, and shControl groups. The gray density was normalized to the mean gray density of GAPDH. (E) Ratio of P-TAU-Y322 and Tau protein expression levels in APPswe293T cells from SV2A, Control, shSV2A, and shControl groups. The gray density was normalized to the mean gray density of GAPDH. For each group of APPswe293T cells, n=3. Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA). *$P < 0.05$.

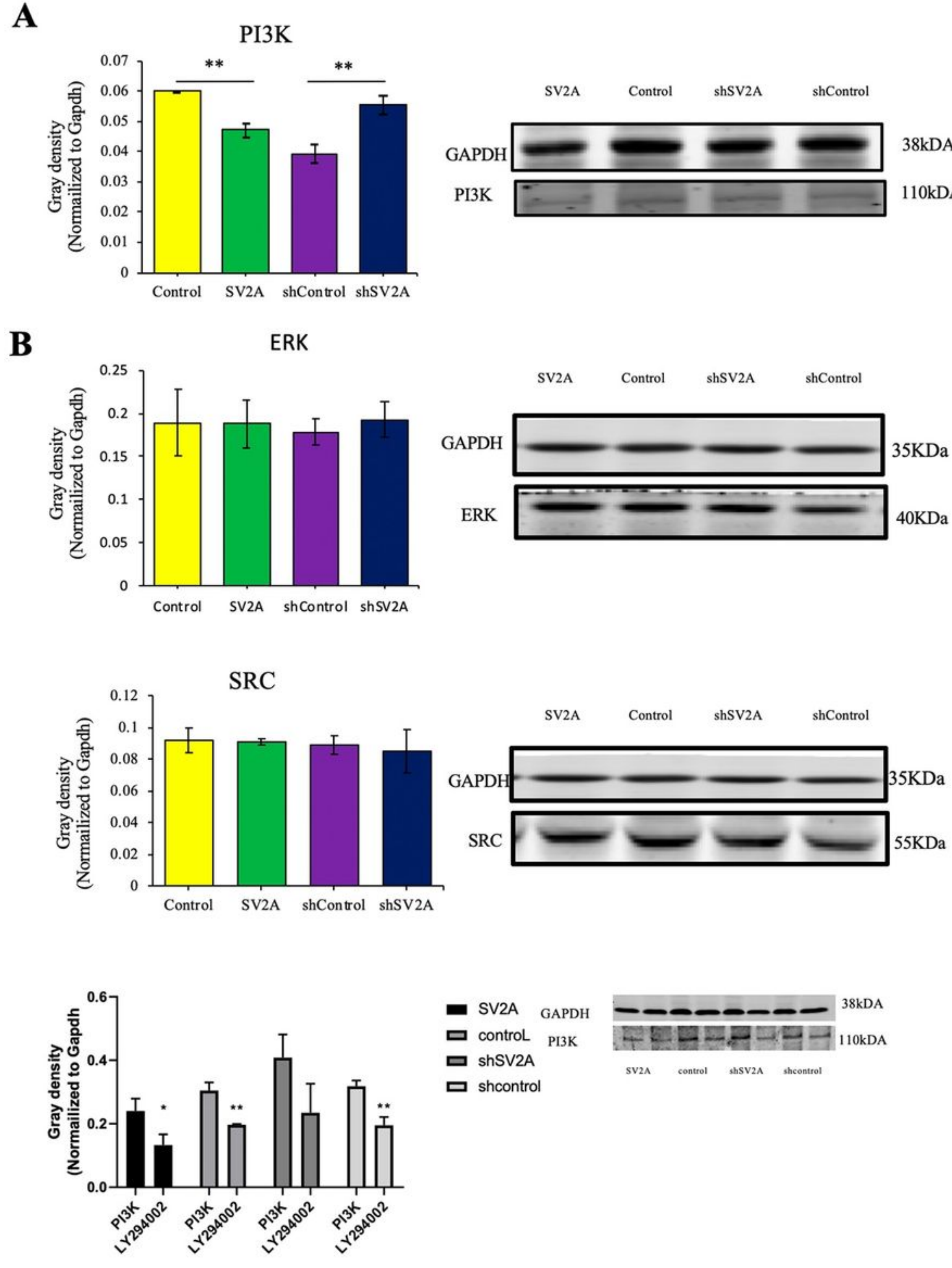


Figure 5

overexpressing of SV2A inhibits PI3K signaling pathway.(A) Western blotting detection of PI3K protein expression levels in APPswe293T cells from Control, SV2A, shControl, and shSV2A groups. The gray density was normalized to the mean gray density of GAPDH. (B) Western blotting detection of ERK protein expression levels in APPswe293T cells from Control, SV2A, shControl, and shSV2A groups. The gray density was normalized to the mean gray density of GAPDH. (C) Western blotting detection of SRC

protein expression levels in APPswe293T cells from Control, SV2A, shControl, and shSV2A groups. The gray density was normalized to the mean gray density of GAPDH. (D) Comparison of Western blotting detection of PI3K protein expression levels in APPswe293T cells and APPswe293T cells with LY294002 (PI3K signaling pathway inhibitor) from SV2A, Control, shSV2A, and shControl groups. The gray density was normalized to the mean gray density of GAPDH. For each group of APPswe293T cells, n=3. Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA). *P < 0.05, **p < 0.01.

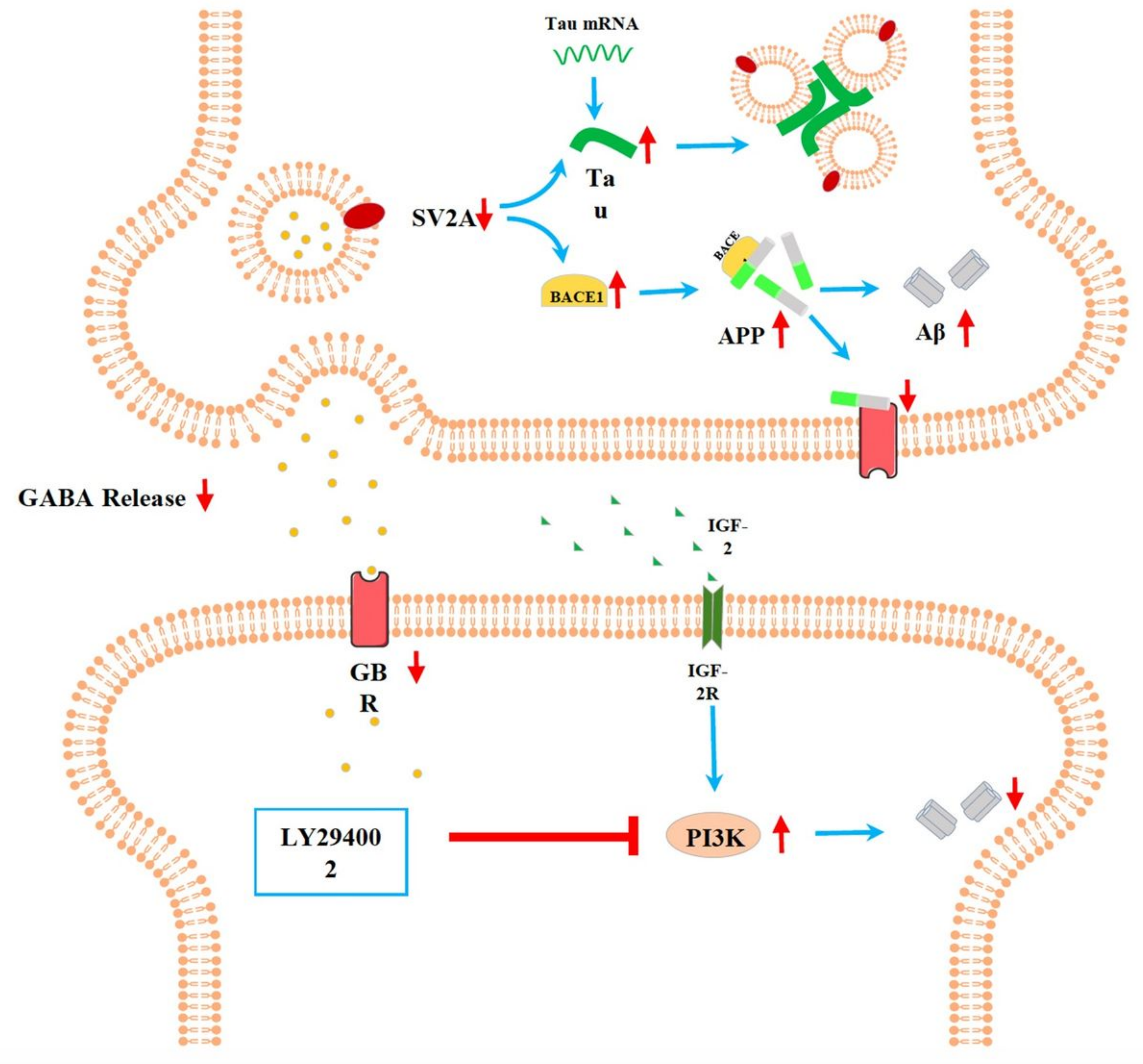


Figure 6

Schematic diagram showing that the deficiency of SV2A up-regulates Tau expression to reduce the inhibition of the impairment of synaptic vesicle information transmission, up-regulates BACE1 expression and inhibits GBR stabilizing APP on the cell surface thus promotes the proteolysis of APP to Aβ. SV2A also regulates PI3K pathway. Via the above 3 ways does SV2A regulates AD-related proteins.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.docx
- FigureS1.docx
- SupplementaryMaterial.pdf
- SupplementaryMaterial.pdf
- AnimailsEthical.pdf
- AnimailsEthical.pdf