Neurobiology of Disease 127 (2019) 410–418

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Amyloid beta-mediated KIF5A deficiency disrupts anterograde axonal mitochondrial movement

Qi Wang[a,b], Jing Tian[b], Hao Chen[b], Heng Du[a,b,*], Lan Guo[b,*]

[a] AD Center, Department of Neurology, Qianfoshan Hospital Affiliated to Shandong University, Jinan, Shandong 250014, China
[b] Department of Biological Sciences, The University of Texas at Dallas, 800 W. Campbell Road, Richardson, TX 75080, USA

ARTICLE INFO

Keywords:
Amyloid beta
Axonal mitochondrial motility
KIF5A
Anterograde transport
Alzheimer's disease

ABSTRACT

Mitochondria are crucial organelles for neurophysiology and brain mitochondrial defects constitute a characteristic of Alzheimer's disease (AD). Impaired axonal mitochondrial traffic, especially the anterograde axonal mitochondrial transport is a pronouncing mitochondrial defect that underlies synaptic failure in AD-related conditions. However, the detailed molecular mechanisms of such axonal mitochondrial abnormality have not been fully understood. KIF5A is a key isoform of kinesin-1, which is a key molecular machinery in facilitating anterograde axonal mitochondrial transport. In this study, we have determined a downregulation of KIF5A in postmortem AD temporal lobes. Further experiments on amyloid beta (Aβ)-treated primary neuron culture and 5 × FAD mice suggest a close association of Aβ toxicity and KIF5A loss. Downregulation of KIF5A mimics Aβ-induced axonal mitochondrial transport deficits, indicating a potential role of KIF5A deficiency in AD-relevant axonal mitochondrial traffic abnormalities. Importantly, the restoration of KIF5A corrects Aβ-induced impairments in axonal mitochondrial transport, especially the anterograde traffic, with little or no impact on retrograde axonal mitochondrial motility. Our findings suggest a novel KIF5A-associated mechanism conferring Aβ toxicity to axonal mitochondrial deficits. Furthermore, the results implicate a potential therapeutic avenue by protecting KIF5A function for the treatment of AD.

1. Introduction

Kinesin superfamily proteins (KIFs) and cytoplasmic dynein are the major types of microtubule-dependent motor proteins, which are essential molecular machineries for intracellular transport (Hirokawa et al., 2009). The directionality of intracellular transport of cargoes is decided by the interaction of microtubule “rails” with KIFs or dynein. Active KIF- and dynein- driven intracellular transport is extremely critical for the maintenance of function and morphology of cells, especially for those with cellular subcompartments in distance from the soma, for example neurons (Hollenbeck and Saxton, 2005; Pilling et al., 2006; Saxton and Hollenbeck, 2012; Vagnoni and Bullock, 2018). Axons are long-stretching processes stemming from the neuronal soma. Given their unique morphological features axons thus have an intensive demand for a normal axonal transport of proteins, RNAs as well as membrane organelles such as mitochondria.

Mitochondria are the major energy provider. Moreover, neuronal mitochondria serve as a critical calcium reservoir that helps to maintain intraneuronal calcium homeostasis (Du et al., 2008; Sui et al., 2018), particularly in axons where smooth endoplasmic reticulum (SER) is scant (Luarte et al., 2018). Therefore, active transport of axonal mitochondria is a prerequisite for synaptogenesis and synaptic transmission. As a result, defected mitochondrial delivery has been linked to synaptic failure and axonal degeneration in neurodegenerative disorders including Alzheimer's Disease (AD) (Correia et al., 2016; Du et al., 2010; Guo et al., 2013; Wang et al., 2010). AD is a lethal neurodegenerative disorder characterized by progressive cognitive decline majorly attacking the aging population (Cummings, 2004). Neuronal mitochondrial deficits including compromised mitochondrial bioenergetics, lowered mitochondrial calcium retention capacity, as well as deregulated mitochondrial dynamics and motility, are pronounced early AD pathologies exacerbated with the progress of this neurological disorder (Reddy et al., 2012; Swerdlow, 2018). AD mediators including Amyloid beta (Aβ) (Calkins and Reddy, 2011; Rui and Zheng, 2016), Tauopathy (Llorens-Martin et al., 2011; Rodriguez-Martin et al., 2016) and presenilin-1 (PS1) mutant (Pigino et al., 2003) alone or in combination are proposed to disrupt axonal mitochondrial transport. The influence of Aβ toxicity on axonal mitochondrial movement has been heavily investigated, although the detailed molecular mechanisms connecting Aβ toxicity and axonal mitochondrial moving defects still

* Corresponding authors at: Department of Biological Sciences, The University of Texas at Dallas, 800 W. Campbell Road, Richardson, TX 75080, USA.
E-mail addresses: heng.du@utdallas.edu (H. Du), lan.guo@utdallas.edu (L. Guo).

https://doi.org/10.1016/j.nbd.2019.03.021
Received 15 December 2018; Received in revised form 18 March 2019; Accepted 21 March 2019
Available online 25 March 2019
0969-9961/

remain unclear. Intriguingly, many groups including us have observed an increased vulnerability of anterograde axonal mitochondrial transport in comparison with their retrograde movement in Aβ-rich milieu (Calkins et al., 2011; Calkins and Reddy, 2011; Du et al., 2010; Guo et al., 2013). Because KIFs are key effectors for anterograde transport (Sheng and Cai, 2012), such finding seems to implicate a potential involvement of impaired KIF-driven axonal mitochondrial delivery in AD-related conditions. It should be noted that among the known KIFs, kinesin-1 (KIF5) including its three isoforms KIF5A, 5B and 5C is critical for anterograde mitochondrial transport in neurons (Sheng and Cai, 2012; Tanaka et al., 1998). Importantly, KIF5A and KIF5C are exclusively expressed in neurons (Kanai et al., 2000). However, current researches on the expression levels of KIF5s in AD-related conditions show inconsistent results (Borysov et al., 2011; Hares et al., 2017; Sepulveda-Falla et al., 2014; Sherman et al., 2016; Stokin et al., 2005).

Here, we assessed the expression levels of KIF5s and found a substantial reduction in the expression levels of KIF5A with a moderate decrease of the expression level of KIF5B in the temporal lobes from postmortem AD brains. In contrast, the expression levels of KIF5C, dynein cytoplasmic heavy chain (DCHC), and the adaptor proteins including Miro1 and syntabulin were relatively preserved in AD subjects. The reduction of KIF5A in an AD mouse model mimicking AD brain amyloidopathy (5 × FAD mice) and Aβ-treated neurons further suggests a potential association of KIF5A loss in neurons with Aβ toxicity. Downregulation of KIF5A in neurons induced an Aβ-like effect on axonal mitochondrial motility. Further experiments showed that the restoration of KIF5A in neurons significantly rescued anterograde axonal mitochondrial transport from Aβ toxicity along with attenuated neuronal stress including oxidative stress and loss of postsynaptic density protein 95 (PSD 95) and synaptophysin. Therefore, Aβ-mediated KIF5A downregulation is a potential mechanism of axonal mitochondrial movement deficits in AD-related conditions, suggesting KIF5A as a promising therapeutic target for AD mitochondrial dysfunction.

2. Results

2.1. Reduced KIF5A expression in AD subjects

To examine the expression levels of KIF5s, we subjected protein extracts from the temporal lobes of nonAD and AD subjects (Table 1) to immunoblotting. The temporal lobe is an AD sensitive brain region, which shows changes at the very early stage of AD (Frisoni et al., 1996). AD cases demonstrated a remarkable reduction in the expression levels of KIF5A in comparison with the nonAD controls (Fig. 1A and B). In contrast, AD cases showed a moderate decrease in KIF5B levels with a slight reduction of KIF5C expression (Fig. 1A and B). Interestingly, the examination of KIF5s in the cerebellum, a relatively unaffected brain region in AD, revealed indiscernible changes of KIF5A or KIF5B expression between AD and nonAD cases (Fig S1), suggesting a close association of KIF5A loss with AD pathology. Mitochondrial transport is reliant on the coordination of anterograde and retrograde effectors. We thus measured the expression levels of dynein cytoplasmic heavy chain (DCHC) and found little or no change in AD cases (Fig. 1A and B). Lastly, previous studies have identified that syntabulin (Cai et al., 2005) and Miro1 (Saotome et al., 2008; Wang and Schwarz, 2009) are two critical adaptor systems attaching kinesin to mitochondria in neurons. In this regard, we further examined the expression levels of syntabulin and Miro1 in AD brains by immunoblotting. AD brains showed a moderate decrease of Miro1 levels in proportion to the reduction of Tom40 content (Fig. 1A and B). Because Miro1 predominantly resides on outer mitochondrial membrane (OMM) (Fransson et al., 2003), these results implicate an association of Miro1 loss and decreased mitochondrial mass in AD. However, there is no significant change in the expression levels of syntabulin in AD cases (Fig. 1A and B). Given the exclusive abundance of KIF5A in neural cells as well as KIF5A's role in driving anterograde mitochondrial movement, our findings echo with the previously reported selective damage of anterograde mitochondrial transport in AD-relevant conditions (Calkins et al., 2011; Calkins and Reddy, 2011; Du et al., 2010; Guo et al., 2013).

2.2. KIF5A loss is associated with Aβ toxicity

Aβ is a key mediator of AD. To determine whether KIF5A loss in AD brains is a result of Aβ toxicity, we examined KIF5A expression levels in the neocortex from 5 × FAD mice at 8 and 12 months old, which mimic brain amyloidopathy at middle and late stages of AD, respectively (Beck et al., 2016; Eimer and Vassar, 2013; Gauba et al., 2019). The analysis of immunoreactive bands showed a remarkable reduction of KIF5A in 5 × FAD mice (Fig. 2A and B). Such change of KIF5A was further confirmed by immunofluorescent staining (Fig. 2C). Of note, although no phenotypic effect on the expression of KIF5C, DCHC or syntabulin was detected, 5 × FAD mice showed a moderate decrease in the expression levels of KIF5B, but the reduction of KIF5B expression in 5 × FAD mice at both tested ages was much less rigorous as that of KIF5A (Fig. 2A and B). The expression levels of Miro1 and Tom40 were relatively unchanged in the neocortex from 5 × FAD mice until at the mouse age of 12 months old (Fig. 2A and B). The recapitulation of AD-relevant KIF5A changes in the Aβ-overproducing mice especially at an older age seem to imply an Aβ effect on the expression levels of KIF5A, which was further confirmed by exposing primary cultured neurons to oligomeric Aβ1–42 at 1 μM for an incubation of 24 h (Fig. 2D) to mimic oligomeric Aβ neurotoxicity in late-stage AD (Rui and Zheng, 2016; Yang et al., 2017). Of note, except for the loss of Miro1, the expression levels of other KIF5s, DCHC, and syntabulin were largely preserved in Aβ-insulted primary neuron culture (Fig. 2D). These results serve as direct evidence that KIF5A loss in AD brains is, at least in part, associated with Aβ toxicity.

2.3. KIF5A downregulation mediates Aβ-like effect on axonal mitochondrial transport

To verify the influence of KIF5A deficiency on axonal mitochondrial

Table 1
Human brain tissues information.

Clinical Dx	Case number	Gender	Age	PMI(Hr)	Braak
nonAD	36359	M	84	24	IV
nonAD	42990	F	84	14	I
nonAD	45329	M	78	21	I
nonAD	39146	F	67	12	II
nonAD	46202	M	77	20	II
AD	46090	F	75	27	VI
AD	46121	F	74	16	VI
AD	46991	M	62	23	V
AD	47586	F	78	18	V
Mean ± SEM(nonAD)		2F/3M	78 ± 3.11	18.2 ± 2.24	
Mean ± SEM(AD)		3F/1M	72.25 ± 3.52	21 ± 2.48	

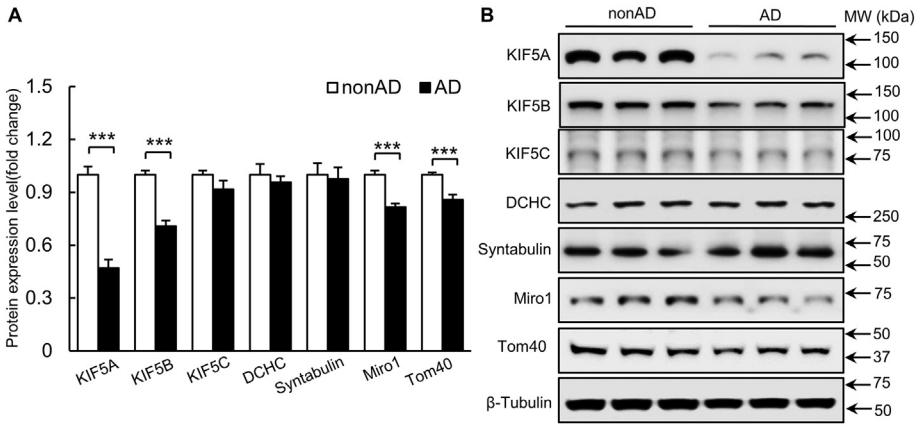

Fig. 1. Reduced KIF5A expression level in AD subjects. (A) Western blot analysis of protein expression levels in protein extracts from the temporal lobes of patients with AD and nonAD control donors. β-tubulin was used as a loading control for KIF5A, KIF5B, KIF5C, DCHC, Syntabulin, Miro1 and Tom40 level measurement. Unpaired Student's *t*-test. $n = 9–12$ from 4 CE subjects and 5 nonAD controls. Error bars represent s.e.m. (B) Representative immunoreactive bands.

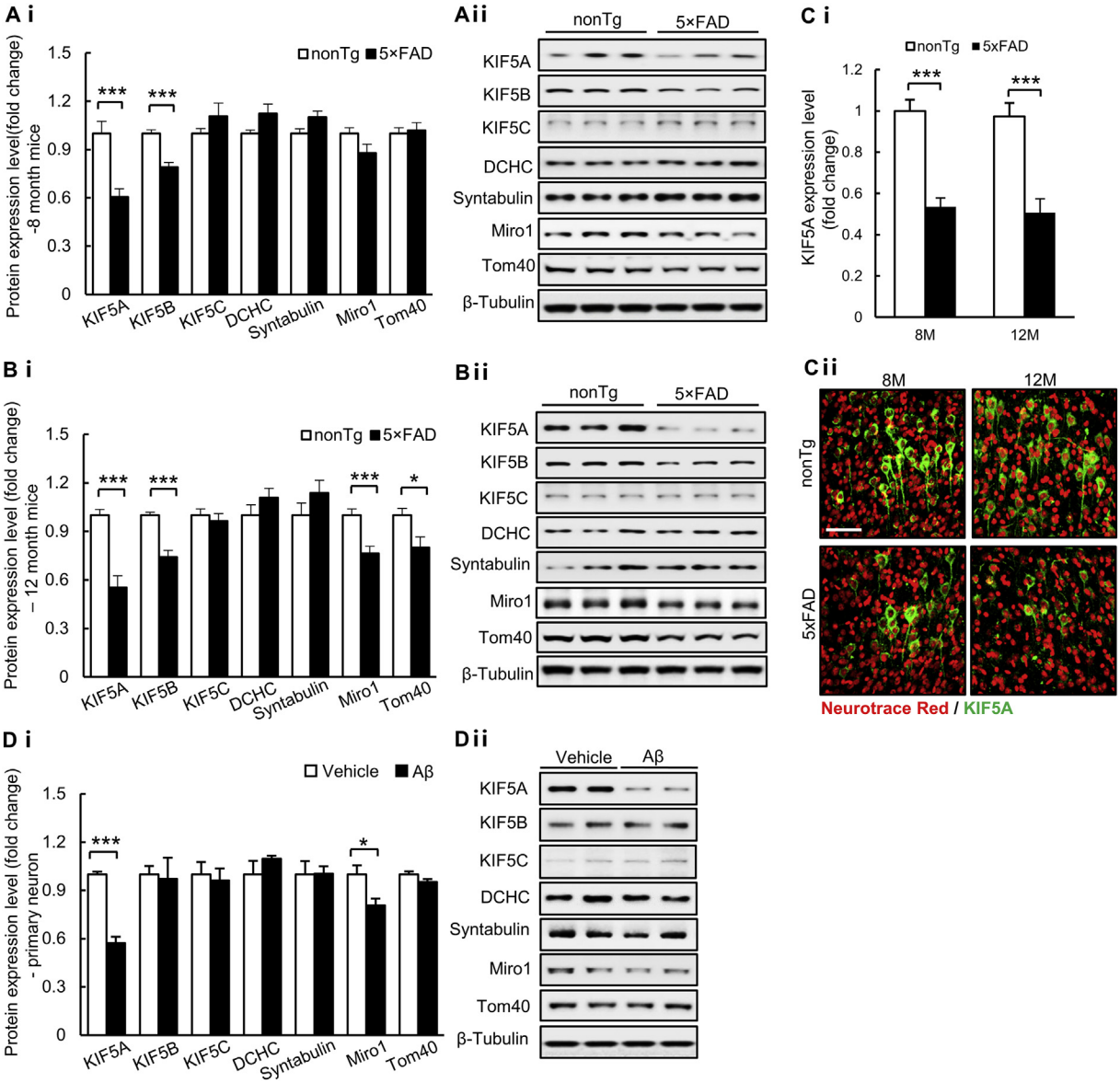

Fig. 2. KIF5A loss is associated with Aβ toxicity. (A) Western Blot analysis of protein expression levels in neocortex from 8 months old 5 × FAD mice. Aii shows representative immunoreactive bands. Unpaired Student's *t*-test. $n = 10–15$ samples. (B) Western Blot analysis of Protein expression levels in neocortex from 12 months old 5 × FAD mice. Bii shows representative immunoreactive bands. Unpaired Student's *t*-test. $n = 9–16$ samples. (C) Immunostaining of KIF5A on brain sections from 8 months and 12 months old 5 × FAD mice. Cii are representative images of Neurotrace Red (red, neurons) and KIF5A (green). Unpaired Student's *t*-test. $n = 20–26$. Scale bar = 50 μm. (D) Protein expression levels in primary cultured neurons treated with 1 μM Aβ. Unpaired Student's *t*-test. $n = 9–10$. Error bars represent s.e.m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

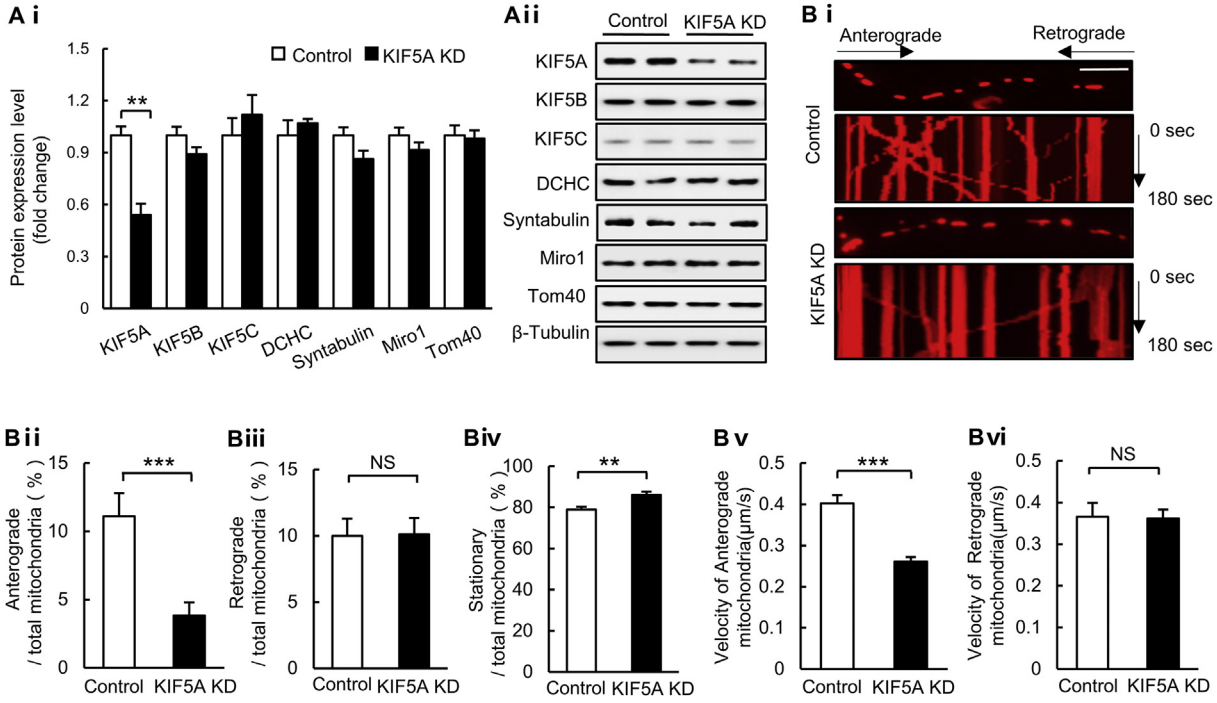

Fig. 3. KIF5A loss mediates Aβ-like effect on axonal mitochondrial transport. (A) Western Blot analysis of protein expression levels in primary cultured neurons infected with lentivirus carrying KIF5A shRNA (KIF5A KD, KIF5A knockdown) or the lentivirus carrying nonTarget shRNA (Control). Unpaired Student's *t*-test. n = 8–10 samples. (B) The effects of KIF5A knockdown on axonal mitochondrial movement. Bi shows images of axonal mitochondria in the top portion of the panel and kymographs in the lower panel were generated from the live imaging movies. In the kymographs, the X axis represents the mitochondrial position and the Y axis is time. Vertical white lines represent stationary mitochondria and diagonal lines represent moving mitochondria. Anterograde movements are from left to right, retrograde movements are reversed. Scale bar = 10 μm. Percentages of anterograde-transported (Bii), retrograde-transported (Biii) and stationary (Biv) mitochondria were compared with those of total mitochondria, respectively. Control, n = 8 neurons; KIF5A KD, n = 14 neurons. Average anterograde (Bv) and retrograde (Bvi) transport velocity of movable mitochondria (μm/s) is shown. n = 15–59 mitochondria. Unpaired Student's *t*-test. Error bars represent s.e.m.

motility, we genetically down regulated the expression of KIF5A in primary cultured mouse neurons by using specific KIF5A shRNA. Nontarget shRNA was used as control. Densitometry analysis showed a remarkable reduction of KIF5A levels in KIF5A knockdown neurons (KIF5A KD, Fig. 3A). Further analysis suggested that KIF5A deficiency had little effect on the expression levels of other KIF5s, DCHC, syntabulin or Miro1 (Fig. 3A). The results indicate the specificity of KIF5A shRNA and uncoupled regulation of KIF5s. To examine axonal mitochondrial transport, mitochondria were visualized by mitoDsRed and the movement of axonal mitochondria in KIF5A-deficient as well as the control neurons was observed under fluorescent microscope. As expected, in comparison with their control counterparts, KIF5A-deficient neurons exhibited substantially impaired anterograde movement, which was demonstrated by decreased percentage of mitochondria under anterograde transport as well as reduced mitochondrial anterograde moving velocity (Fig. 3Bi, ii and v). Of note, the retrograde movement of axonal mitochondria was largely preserved in KIF5A shRNA-treated neurons (Fig. 3Bi, iii and vi). As a result of blunted anterograde mitochondrial transport, KIF5A deficient neurons had significantly increased percentage of stationary mitochondria (Fig. 3Bi and iv). Importantly, such KIF5A loss-induced axonal mitochondrial movement abnormalities are in similar patterns as those observed in Aβ-treated neurons, which is supported by previous reports (Guo et al., 2013) (Du et al., 2010) as well as our further examination (Fig. 4). Thus, it seems that Aβ-induced KIF5A deficiency is closely associated with defected axonal mitochondrial movement in AD-related conditions.

2.4. KIF5A restoration rescues axonal mitochondrial transport from Aβ toxicity

To address the role of KIF5A deficiency in axonal mitochondrial transport deficits in an Aβ-rich environment we examined whether the Aβ-mediated axonal mitochondrial motility deregulation can be mitigated by KIF5A restoration. To this end, we overexpressed KIF5A in mouse neurons by using a lentivirual delivery system. KIF5A overexpressing neurons demonstrated a substantial elevation in KIF5A expression levels (Fig. 4A) with no significant impact on the expression levels of other KIF5s, DCHC, Syntabulin, or Miro1. The control and KIF5A overexpressing (KIF5A OE) neurons were exposed to a treatment with oligomeric Aβ at 1 μM for 24 h. Of note, the up-regulation of KIF5A expression conferred resistance to neurons against Aβ-induced KIF5A loss (Fig. 4B). Further axonal mitochondrial motility assays showed that KIF5A overexpression substantially attenuated the Aβ-mediated anterograde axonal mitochondrial transport defects by rescuing the percentage of mitochondria under anterograde transport (Fig. 4Ci and ii) and the moving velocity (Fig. 4Ci and v), as well as the percentage of stationary mitochondria (Fig. 4Ci and iv). It should be noted that the overexpression of KIF5A did not affect the retrograde movement of axonal mitochondria in the presence or absence of Aβ (Fig. 4Bi, iii and vi). Put together, our results support the contribution of Aβ-induced KIF5A deficiency to axonal mitochondrial motility deficits in AD-related conditions.

2.5. KIF5A restoration protects neurons from Aβ-induced neuronal stress

In view of the deleterious impact of KIF5A deregulation on axonal cargo transport and supply of synapses, previous studies have linked KIF5A loss-of-function to several neuronal injury (Karle et al., 2012; Lo

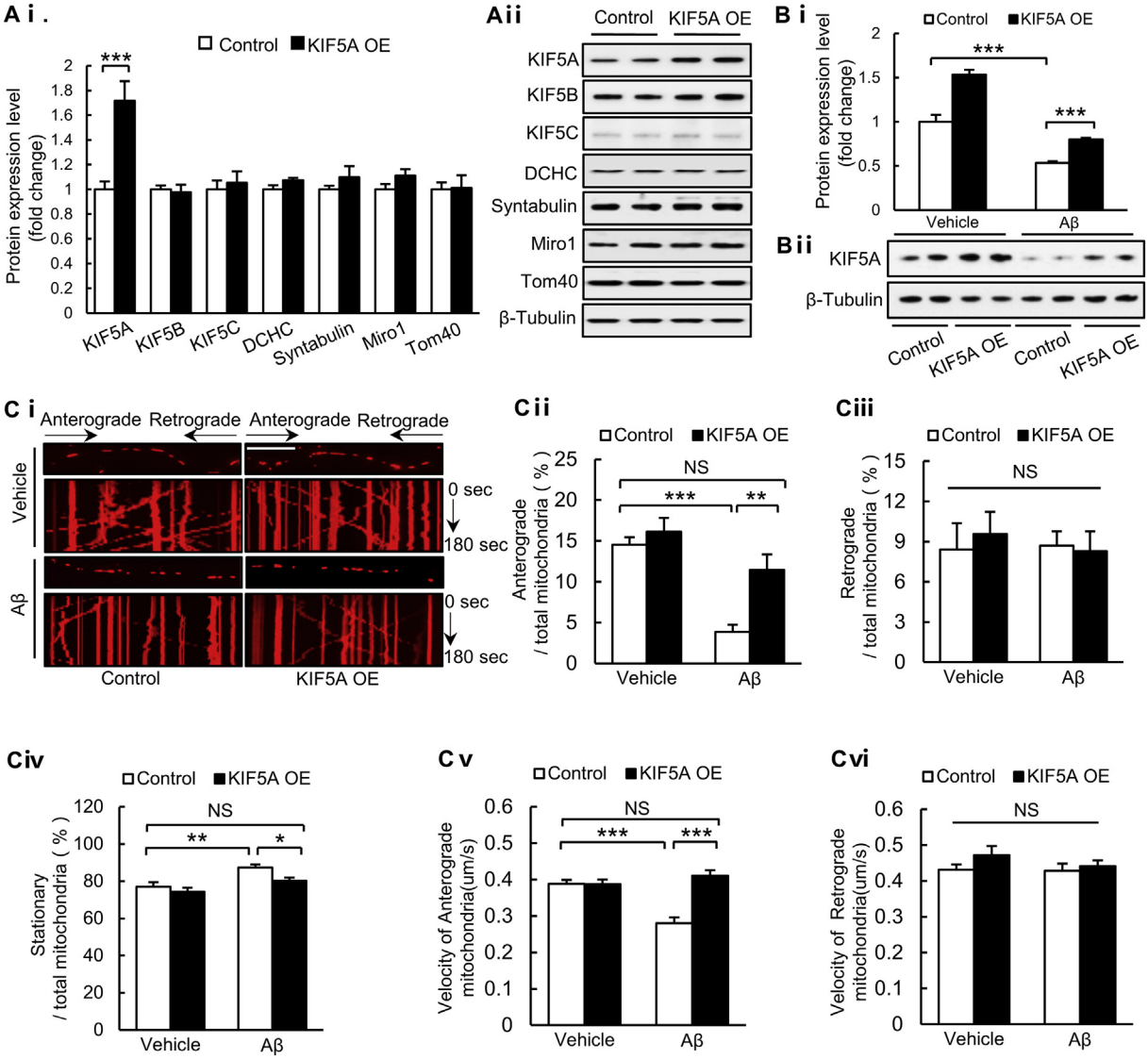

Fig. 4. KIF5A restoration rescues axonal mitochondrial transport from Aβ toxicity. (A) Protein expression level in KIF5A overexpressing neurons. Unpaired Student's *t*-test. $n = 8–11$ samples. (B) KIF5A overexpression attenuated Aβ-induced KIF5A reduction in neurons. Unpaired Student's *t*-test. $n = 9$ per group. (C) KIF5A overexpression protected mitochondrial movement against Aβ toxicity. Scale bar = 10 μm. One-way ANOVA followed by Bonferroni post hoc analysis was used for the analysis. $n = 10–13$ neurons in Cii,iii and iv. $n = 109–276$ mitochondria in Cv and Cvi. Error bars represent s.e.m.

Giudice et al., 2006). Therefore, it would be of interest to determine whether KIF5A restoration also protects neurons from oligomeric Aβ-induced damages along with mitigated axonal mitochondrial transport as seen in Fig. 4. As expected, KIF5A overexpression substantially attenuated oligomeric Aβ treatment-mediated excess neuronal reactive oxygen species (ROS) production demonstrated by decreased DCF intensity (Fig. 5A) as well as lipid oxidation exhibited by the staining of 4-hydroxynonenal (4-HNE) (Fig. 5B). Moreover, the protective effect of KIF5A overexpression against oligomeric Aβ-induced synaptic loss was determined by the observation of preserved content of synaptic proteins including PSD 95 (postsynaptic marker, Fig. 5C) and synaptophysin (presynaptic marker, Fig. 5D). These results support a protective role of KIF5A restoration against neuronal oxidative stress and synaptic failure in an Aβ-enriched environment.

3. Discussion

KIF5s are proposed to be key molecular factors for mitochondrial transport in neurons (Sheng and Cai, 2012). Distinct from the ubiquitous distribution of KIF5B, KIF5A and C have a selective abundance in neurons, which underscores the importance of KIF5A and C in neural cells (Frederick and Shaw, 2007). Moreover, KIF5A seems to be particularly critical for intra-neuronal mitochondrial transport. This is based on the facts that (1) KIF5A demonstrates exclusive high abundance in neurons (Hirokawa et al., 2010; Hirokawa and Takemura, 2005; Kanai et al., 2000); (2) KIF5A dysfunction is unlikely to be compensated by other KIF5s (Campbell et al., 2014); and (3) dominant negative mutations in KIF5A gene have medical relevance (Crimella et al., 2012; Reid et al., 2002). Given the critical role of mitochondria in maintaining axonal physiology (Sheng and Cai, 2012), it is not surprising that KIF5A loss may accompany defected axonal mitochondrial transport as we found in this study, resulting in the failure of mitochondrial delivery to fuel synapses. Importantly, the reduced KIF5A expression and the relative preserved dynein perfectly match with the reported selective vulnerability of anterograde axonal mitochondrial transport in AD-related pathological setting. Therefore, KIF5A dysfunction seems to be a characteristic AD-relevant change in neurons. However, it should be noted that mitochondria are not the only cargo in KIF5s-driven axonal transport. KIF5s are also responsible for the delivery of crucial proteins including AD-relevant amyloid beta precursor protein (APP) (Kamal

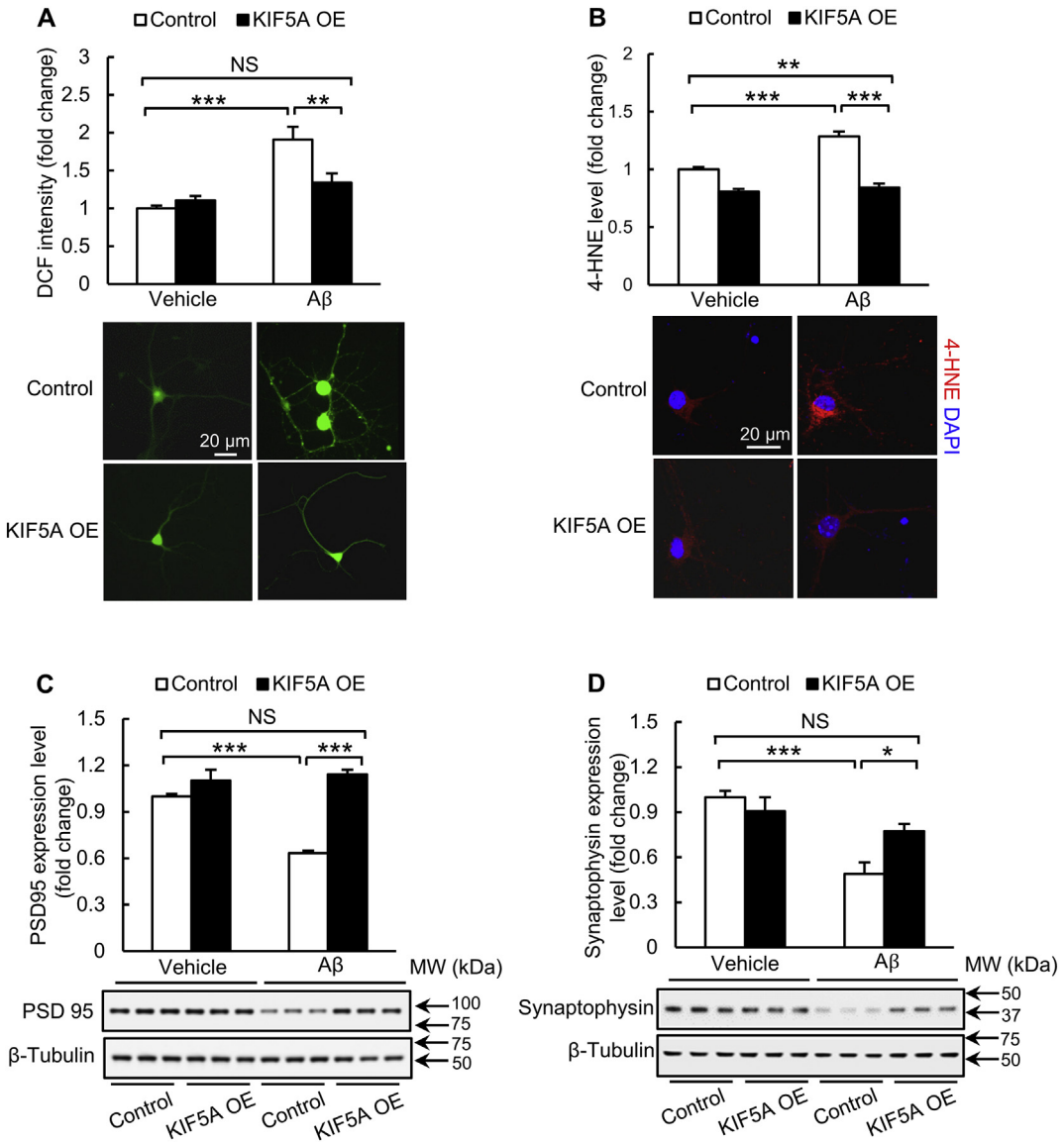

Fig. 5. KIF5A restoration protects neurons from Aβ-induced oxidative stress and synaptic loss. (A) Analysis of neuronal ROS level using DCF staining. One-way ANOVA followed by Bonferroni post hoc analysis. n = 10–22. (B) 4-HNE level measurement in control and KIF5A overexpressing neurons with treatment of vehicle or Aβ. One-way ANOVA followed by Bonferroni post hoc analysis. n = 11 samples. (C and D) Western Blot analysis of expression level of synapse markers including PSD95 (C) and synaptophysin (D). One-way ANOVA followed by Bonferroni post hoc analysis. n = 9 samples. Error bars represent s.e.m.

et al., 2000). Previous studies have suggested that APP might serve as an adaptor for kinesin-1 (that is, KIF5s) (Gunawardena and Goldstein, 2001). Interestingly, Stokin and the colleagues have shown that genetic loss of kinesin-1 expression significantly enhances the production of Aβ40 and Aβ 42 in transgenic mice with the overexpression of APP (Stokin et al., 2005). They further suggest that such kinesin-1-related defects hamper axonal transport and augment the production of Aβ via the proteolytic processing of APP, culminating in brain amyloidosis in AD brains. Moreover, a very recent study has reported an inverse relationship of KIF5A expression with the levels of APP and soluble Aβ (Hares et al., 2017). Therefore, in combination with our observations of the deleterious effect on axonal mitochondrial movement, altered expression levels of KIF5s may play multifaceted roles in promoting several key AD pathologies. In this context, it has raised an intriguing question that whether KIF5A deficiency is a cause or effect of AD. In our study we have shown a direct impact of Aβ on KIF5A expression levels evidenced by the observations of KIF5A loss in Aβ-treated neurons and 5 × FAD mice. Such finding seems to implicate a loop that Aβ triggers KIF5A deregulation, which further enhances the production and accumulation of Aβ in axons. Notably, previous studies have shown that many neuronal stressors for example oxidative stress may cause the downregulation of KIFs (Redondo et al., 2015). Given the facts that neuronal oxidative stress constitutes an early characteristic of AD brains and that AD brain oxidative stress is closely associated with mitochondrial dysfunction (Reddy et al., 2012; Swerdlow, 2018), we thus propose a vicious cycle involving Aβ, mitochondria and KIF5A in the development of axonopathy in AD. Our hypothesis seems to be further supported by our observations of the concurrence of rescued mitochondrial motility, attenuated neuronal oxidative stress and loss of synaptic content in Aβ-treated KIF5A-overexpressing neurons. Future studies on this topic will help us to address this question.

A critical issue that merits discussion is KIF5A downregulation in AD-relevant pathological settings. A very recent study reported an inconsistent result by showing increased KIF5A expression levels in postmortem AD brains (Hares et al., 2017). Hares and the colleagues think that the KIF5A upregulation is a compensatory response to the over-production of Aβ, through which AD brains control Aβ generation and accumulation. This finding is not supported by our observations

with Aβ-treated neurons, 5 × FAD mice, or the postmortem AD brains. Indeed, given the complexity of AD pathogenesis, we cannot exclude the existence of such compensatory response. A possible explanation for this discrepancy may be the brain region-specific vulnerability as we used the temporal lobes in this study, which may have different regulation of KIF5A from the frontal lobes as used in Hares' study. Further comprehensive mapping of KIF5A expression in different AD brain regions is thus called for. Nevertheless, the restoration of axonal mitochondrial transport by KIF5A overexpression along with the crucial role of KIF5A in neuronal mitochondrial transport (Campbell et al., 2014) serve as strong evidence that KIF5A loss probably due to Aβ toxicity is a major causative factor for axonal mitochondrial motility impairment in AD-related conditions. Kinesin motors are long-lived proteins, and recycled to participate in multiple rounds of transport (Blasius et al., 2013; Brown et al., 2005). Due to the irrelevance of proteasomal degradation pathway in the regulation of Kinesin in neurons (Blasius et al., 2013), we thus propose that loss of KIF5A may potentially result from compromised KIF5A expression at pre- and/or post-transcription levels in response to AD-relevant pathology. Although we do not have a clear answer to this question at this moment, our speculation is supported by previous studies showing that oxidative stress downregulates KIF5A at both gene and protein expression levels (Redondo et al., 2015). Of note, we also found a moderate reduction of KIF5B expression levels in AD patients and 5 × FAD mice. Different from neuron specific distributions of KIF5A and 5C, KIF5B is ubiquitously expressed in all cell types (Kanai et al., 2000) including neurons (Tanaka et al., 2011). In this regard, the loss of KIF5B in AD patients and 5 × FAD mice might involve the changes in non-neuronal cells, especially glial cells. This hypothesis is further indirectly supported by our findings of preserved KIF5B expression in Aβ-treated primary neuron cultures. A further study on cell type-specific changes of kinesin-1 isoforms will help to answer this question. Moreover, it is also possible that the discrepancy of KIF5B expression between in vitro and in vivo settings is a result of neuronal response to chronic insults in patient brains.

Lastly, increasing evidence has suggested that the disruption of axonal mitochondrial transport is likely a synergistic effect of Aβ toxicity and Tau abnormalities in AD (Correia et al., 2016). Mitochondrial dysfunction, impaired microtubule “rails” due to altered α-tubulin acetylation and Tau interaction, as well as KIF disturbances and phosphorylation modification due to Glycogen synthase kinase 3 beta (GSK3B) dysregulation are known contributing factors (Correia et al., 2016; Eckert et al., 2014; Morel et al., 2010). The limitation of our study is the predominant focus on KIF5A deficiency and this observation in an Aβ-rich milieu. In fact, a previous study also found oligomeric Aβ induces a reduction in the light chain of Kinesin (KLC-1) and causes intracellular accumulation of APP, resulting in tau pathology (Sherman et al., 2016). These results imply multifaceted damages of Kinesin in AD-related conditions. Moreover, we cannot fully rule out the contribution of KIF5B deficits to altered axonal mitochondrial motility especially in a late-stage of AD. These questions along with our current observation lay the groundwork for our future comprehensive investigation of impaired axonal mitochondrial traffic in AD-relevant pathological settings. In summary, in this study we have shown that KIF5A loss is a pronounced change in AD temporal lobes and this change in KIF5A expression is at least in part associated with Aβ toxicity. The correction of axonal mitochondrial transport defects in Aβ-treated neurons suggests that to protect KIF5A function is a potential avenue to ameliorate mitochondrial abnormalities for the treatment of AD.

4. Methods and materials

4.1. Mice

Animal studies were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee (IACUC) and were performed in accordance with the National Institutes of Health guidelines for animal care. 5 × FAD mice overexpress a human form of mAPP-bearing mutations (SwFlLon) and PSEN1 mutations (M146 L and L286 V) linked to familial AD. Mice were allocated randomly to experimental groups for the various experimental measurements based on genotyping. 8 and 12 months old nonTg and 5 × FAD mice of mixed genders were used in the experiments. Day 0–1 pups from C57BL/6 J strain mice were used for primary neuron culture. The investigators performing the experiments did not select the mice allocation. The genotype of all the transgenic animals was confirmed by performing PCR and dot blots for Aβ detection before the experiments. Mice with wrong genotyping were excluded. The number of mice was determined by our previous data and power calculation to ensure the minimal numbers of mice as needed were used in the experiments.

4.2. Human samples

Frozen brain samples and paraffin-embedded brain slices were requested from UT Southwestern Medical Center ADC Neuropathology Core, supported by ADC grant (AG12300) under a protocol approved by The UT Southwestern Medical Center with informed consent from all subjects and the study adhered to the Declaration of Helsinki principles.

4.3. Primary neurons culture

Neurons were cultured as previously described (Sui et al., 2018). Cortices or hippocampi were dissected from Day 0 pups and kept in cold Hank's balanced salt solution (HBSS, Sigma-Aldrich). Tissues were digested in 0.05% trypsin (Sigma-Aldrich) at 37 °C for 15 min to dissociate neurons. Mouse primary neurons were cultured in neuron culture medium (Neurobasal A with 2% B27 supplement, 0.5 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin) with an appropriate density on poly-D-lysine- (Sigma-Aldrich) coated plastic or glass bottom culture plates (Corning). Mitochondria motility measurement was conducted on neurons with density of $0.1 \times 10^5/cm^2$. The samples for Western Blot were collected from neurons with density of $1 \times 10^5/cm^2$.

4.4. Western blot

Samples were prepared in 1× sample loading buffer (50 mM Tris-HCl pH 6.8, 2% SDS, 10%, glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA and 0.02% bromophenol blue) and proteins were separated by SDS-PAGE (12% Nupage Bis-Tris gel, ThermoFisher Scientific; or 6% SDS-PAGE gels), then transferred to a PVDF membrane (Bio-Rad Laboratories) for blotting. After blocking in TBS buffer (20 mM Tris-HCl, 150 mM sodium chloride) containing 5% (wt/vol) nonfat dry milk for 1 h at room temperature, the membrane was then incubated and gently shaken overnight at 4 °C with primary antibodies. This was followed by the incubation with the appropriate secondary antibody for 1 h at room temperature. Images were collected on BioRad Chemidoc Imaging System. Image J software (National Institutes of Health) was used for data analysis. The following primary antibodies were used: KIF5A (Proteintech, #21886-1-AP, 1:1000), KIF5B (Proteintech, #21632-1-AP, 1:1000), KIF5C (Proteintech, #25897-1-AP, 1:1000), Syntabulin (Proteintech, #16972-1-AP, 1:1000), β-tubulin (Proteintech, #66240-1, 1:10000), Tom40 (Proteintech, #18409-1-AP,1:5000), Dynein (Proteintech, #12345-1-AP, 1:2000), Miro1 (Santa Cruze, #Sc-81935, 1:500), PSD95 (CST, #3450, 1:2000), Synaptophysin (CST, #5461, 1:2000). The following second antibodies were used at appropriate dilutions: HRP conjugated Goat anti-mouse IgG (H + L) (Invitrogen, # A16066, 1:4000-10,000) and HRP conjugated goat anti-rabbit IgG (H + L) (Invitrogen, #656120, 1:4000-10,000).

4.5. Immunostaining

Brain tissues from 8 and 12 months old nonTg and 5 × FAD mice were used to perform immunostaining. Citrate buffer was used to perform antigen retrieval followed by blocking in 5% goat serum and 0.3% triton-X for 1 h at RT. Dilution of the following primary antibodies were used: anti-KIF5A (Proteintech, #21886-1-AP, 1:300), anti-4 Hydroxynonenal (abcam, #ab46545, 1:400). Alexa Fluor 488 goat anti-rabbit IgG(H + L) (Life technology, #A11034, 1:400) was used as secondary antibody for 1 h incubation. Neurons were visualized by using NeuroTrace 530/615 Red Fluorescent Nisssl Stain (Invitrogen, #N21482, 1:300) and DAPI (Sigma-Aldrich, #D9542, 300 nM) was used to label nuclei. Tissues were mounted and imaged under Nikon confocal microscope followed by analysis using the NIS Advanced research software.

4.6. Generation of Lentivirus expressing mitochondria targeted DsRed

Mitochondria targeted DsRed (mtDsRed) (Clontech) was inserted into lentivirus vector with human polyubiquitin promoter-C (Addgene). HEK293T cells (ATCC) were transiently cotransfected with lentivirus vector carrying mitochondria targeted DsRed, packaging vector psPAX2 (Addgene) and envelope vector pMD2.G (Addgene) by using calcium phosphate precipitation method. The lentivirus were collected from the culture medium and concentrated with Lenti-X Concentrator (Clontech).

4.7. KIF5A knocking down in mouse primary neurons

Lentivirus-expressing shRNA targeted to mouse KIF5A was packaged with lentivirus shRNA construct (clone TRCN0000428258: 5′-CCGGATGAAGGACAAGCGTAGATACCTCGAGG TATCTACGCTTGTCC TTCATTTTTTTG-3′, Sigma-Aldrich), packaging vector psPAX2 (Addgene) and envelope vector pMD2.G (Addgene). Lentivirus-expressing nontarget shRNA control (SHC002, Sigma-Aldrich) was used as a control. Mouse primary neurons were cultured for 3 days before infection with lentivirus at a multiplicity of infection (m.o.i.) of 5. Neurons were treated and collected for experiments after a further 7 days in culture.

4.8. KIF5A overexpression in mouse primary neurons

Human KIF5A (Addgene, 15239) was inserted into lentivirus vector with human polyubiquitin promoter-C (Addgene). Lentivirus containing KIF5A were packaged and applied on primary neurons similar as shRNA lentiviral vector. Lentiviral vector backbone was used to package control lentivirus. Mouse primary neurons were cultured for 3 days before infection with lentivirus at a m.o.i. of 5. Neurons were collected for experiments after 7 days infection.

4.9. Oligomeric Aβ preparation

Aβ1–42 peptide (GenicBio) was diluted in 1,1,1,3,3,3,-hexafluoro-2-propanol to 1 mM using a glass gas-tight Hamilton syrings with a Teflon plunger. The clear solution was aliquoted in microcentrifuge tubes, and it was dried by vaporation in the fume hood. Peptide film was diluted in DMSO to 5 mM and sonicated for 10 min in bath sonicator. The peptide solution was resuspended in cold HAM'S F-12 to 100 μM and immediately vortexed for 30 s. The solution was then incubated at 4 °C for 24 h. Oligomeric Aβ (1 μM) was applied on neurons for 24 h before cell collection.

4.10. Mitochondria motility measurement

Mouse primary neurons were infected by lentivirus expressing mtDsRed to visualize mitochondria. Time-lapse images were captured every 5 s for a total of 3 min under 40× oil objective on a Nikon inverted fluorescent microscope with an on-stage incubator (37 °C, 5% CO2). A process two to three times longer than other processes stemming from the soma was considered to be an axon. Particles with strong labeling (compared with background) and clear edges confined in axons were considered to be mitochondria. A mitochondrion was considered to be non-mobile if it remained stationary for the entire recording period; movement was counted only if the displacement was more than the length of the mitochondrion (Sui, Tian et al. 2018). Mitochondrial movement toward the distal end of an axon is considered to be anterograde, whereas that toward the proximal end is considered to be retrograde. Kymographs were generated by using Nikon NIS Advanced Research software to demonstrate the overall movement traces of mitochondria during the recording period. The velocity of all mobile mitochondria was measured by using tracking system integrated in Nikon NIS Advanced Research software.

4.11. Measurement of mitochondrial intra-axonal ROS

The Neurons were incubated in the dark at 37 °C with 5 μM CM-H2DCFDA (DCF, Thermo Fisher Scientific, C6827) for 10 min. After two times wash with neuron culture medium, the fluorescence of DCF was observed images using an inverted Nikon inverted microscope with a stage-based chamber (5% CO2, 37 °C). DCF intensity was measured using Nikon NIS Advanced Research software.

4.12. Statistical analysis

Statistical comparisons were performed using GraphPad Prism 5 software. D'agostino and pearson omnibus normality test was used to determine the normality of data. One-way ANOVA followed by Bonferroni post hoc analysis, or unpaired two-way Student's *t*-test were applied in data analysis. Numbers of replicates and *P* value are stated in each figure legend. All data were expressed as the mean ± s.e.m. Significance was concluded when the *P* value was < 0.05. Significance was indicated by symbols including $^{*}P < .05$, $^{**}P < .010$, $^{***}P < .001$. NS (not significant) denotes $P > .05$.

Acknowledgement

This work was supported by research funding from National Natural Science Foundation of China (31271145, 81200847, 81771153), Natural Science Foundation of Shandong Province (JQ201318), Key Research and Development Program of Shandong Province (2018GSF121003), Alzheimer's Association (NIRG-12-242803, AARG-16-442863), and China Scholarship Council (201706220265).

Conflict of interest

The authors have no conflict of interest to claim.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2019.03.021.

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