ACS Chemical Neuroscience

Research Article

pubs.acs.org/chemneuro

Alzheimer's Amyloid-β Oligomers Rescue Cellular Prion Protein Induced Tau Reduction via the Fyn Pathway

Rong-Jie Chen,[†,§] Wei-Wei Chang,[§] Yu-Chun Lin,[§] Pei-Lin Cheng,[‡] and Yun-Ru Chen*[,†,§]

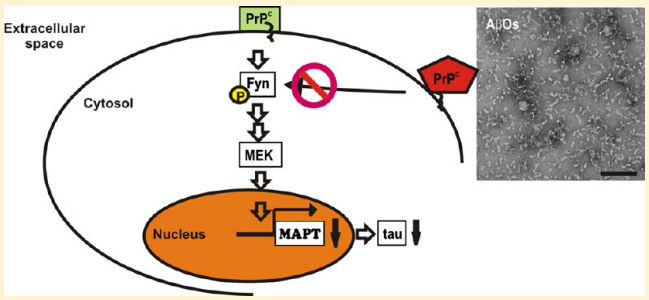
[†]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan

[§]Genomics Research Center, Academia Sinica, Taipei, Taiwan

[‡]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Supporting Information

ABSTRACT: Amyloid-β (Aβ) and tau are the pathogenic hallmarks in Alzheimer's disease (AD). Aβ oligomers are considered the actual toxic entities, and the toxicity relies on the presence of tau. Recently, Aβ oligomers have been shown to specifically interact with cellular prion protein (PrP^C) where the role of PrP^C in AD is still not fully understood. To investigate the downstream mechanism of PrP^C and Aβ oligomer interaction and their possible relationships to tau, we examined tau expression in human neuroblastoma BE(2)-C cells transfected with murine PrP^C and studied the effect under Aβ oligomer treatment. By Western blotting, we found that PrP^C overexpression down-regulated tau protein and Aβ oligomer binding alleviated the tau reduction induced by wild type but not M128V PrP^C, the high AD risk polymorphic allele in human prion gene. PrP^C lacking the Aβ oligomer binding site was incapable of rescuing the level of tau reduction. Quantitative RT-PCR showed the PrP^C effect was attributed to tau reduction at the transcription level. Treatment with Fyn pathway inhibitors, Fyn kinase inhibitor PP2 and MEK inhibitor U0126, reversed the PrP^C-induced tau reduction and Aβ oligomer treatment modulated Fyn kinase activity. The results suggested Fyn pathway regulated Aβ−PrP^C−tau signaling. Overall, our results demonstrated that PrP^C down-regulated tau via the Fyn pathway and the effect can be regulated by Aβ oligomers. Our study facilitated the understanding of molecular mechanisms among PrP^C, tau, and Aβ oligomers.



KEYWORDS: *PrP^C, tau, amyloid-β, oligomers, Fyn, Alzheimer's disease*

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder that affects the elderly. It progressively causes neural damage in the patients' brains leading to dementia. The pathological hallmarks of AD are extracellular amyloid plaques comprising amyloid-β (Aβ) and intracellular neurofibrillary tangles comprising hyperphosphorylated tau protein. Aβ is generated from amyloid-β precursor protein (AβPP), through sequential cleavages of β- and γ-secretases. The major Aβ isoforms are Aβ40 and Aβ42. The soluble oligomeric forms of Aβ42, but not mature fibrils, are considered the actual toxic entities [1] and correlate better with cognitive impairment and synaptic dysfunction.[2] Tau is a microtubule-associated protein that plays a role in axonal transport, neurite outgrowth, and adult neurogenesis.[3] In AD and other tauopathies, tau becomes hyperphosphoryled, which destabilizes the microtubule binding, resulting in tau aggregation.[3] The tau pathology occurs in the later stage of disease progression.[4] Aβ toxicity can be prevented by tau reduction or deficiency as evidenced by transgenic mouse studies.[5] However, the detailed signaling pathways have not been fully understood.

Recently, cellular prion protein (PrP^C) located on the cell surface has been identified as an Aβ oligomer receptor via a high-throughput screening.[6] PrP^C is a GPI-anchored glycoprotein that has been found in the dystrophic neurites and amyloid cores of amyloid plaques in AD brains.[7] The M129V polymorphism in human prion gene *PRNP* is suggested to increase risk toward early onset AD.[8] The Aβ oligomer binding sites on PrP^C were reported to be in the regions of residues 95−110 and 23−27.[6,9] Despite the high-affinity interaction between PrP^C and Aβ oligomers,[9,10] whether PrP^C mediates Aβ oligomer toxicity is still controversial. It has been shown that PrP^C mediates the toxic effect of Aβ oligomers and is required for Aβ oligomer-induced suppression of synaptic plasticity, synapse damage, and neuronal cell death.[6,11] Treatment with PrP^C antibody against the Aβ oligomer binding site prevents the inhibition of long-term potentiation by Aβ oligomers and reverses cognitive deficits in AD transgenic mice.[12] In addition, PrP^C-mediated toxic signaling could be blocked by inhibition of *N*-methyl-D-aspartate receptor (NMDAR) activity.[13] Aβ oligomer binding to PrP^C results in Fyn activation, which leads to phosphorylation of the NR2B subunit of NMDARs.[14]

Received: April 3, 2013
Accepted: June 27, 2013
Published: June 27, 2013

 © 2013 American Chemical Society

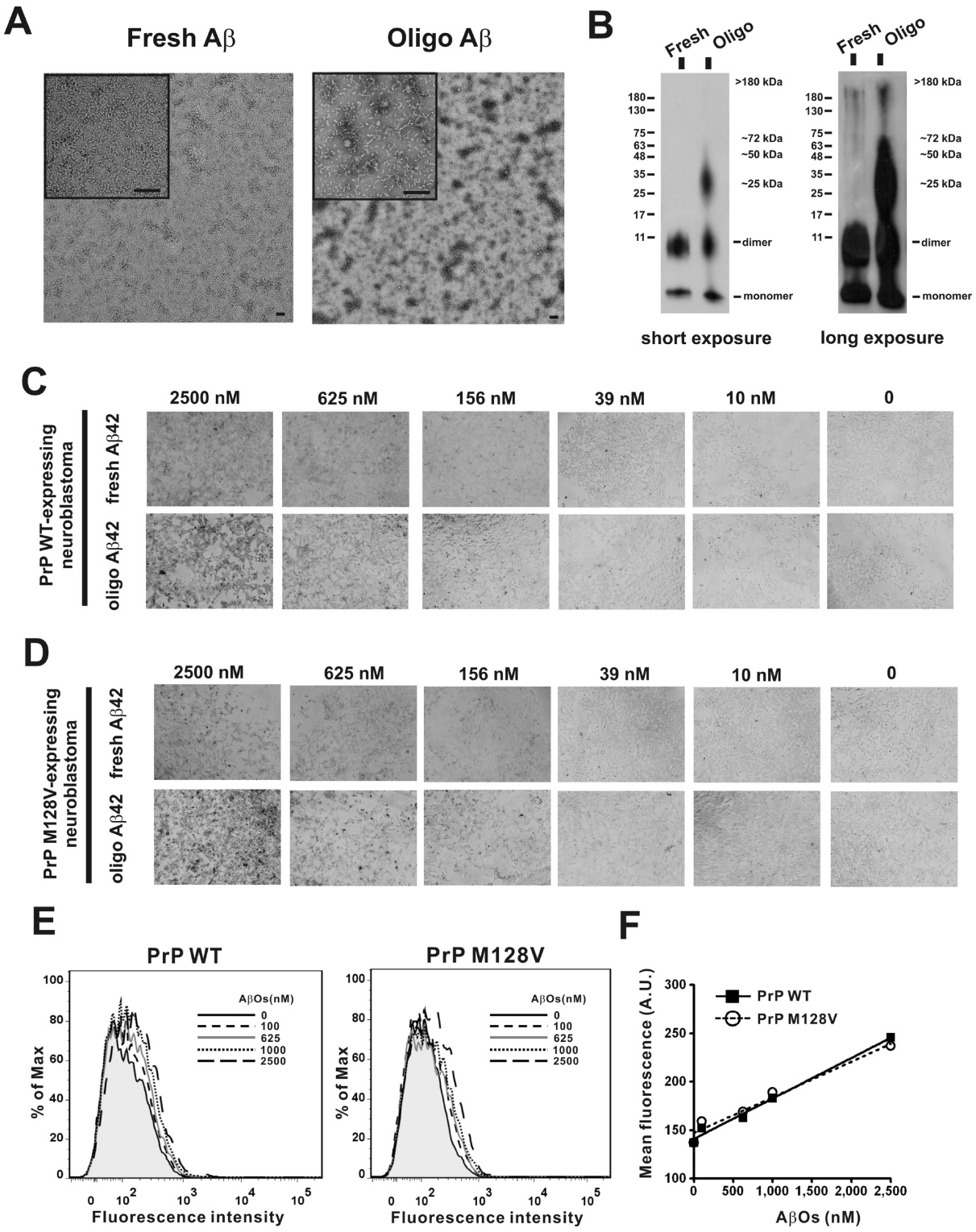


Figure 1. The morphology, assembly, and cellular binding of Aβ oligomers. (A) The morphology of freshly prepared and oligomeric Aβ42 was observed by TEM. Insets are the zoom-in images. Scale bars are 100 nm. (B) The assembly of freshly prepared and oligomeric Aβ42 were examined by Tris-tricine SDS-PAGE and blotted with anti-Aβ antibody 6E10. Molecular mass markers and estimated assembly of Aβ are shown. (C, D) The cellular binding of Aβ42 oligomers to PrP^C expressing cells. Freshly prepared and oligomeric biotinylated Aβ42 (0–2500 nM) were added to PrP^C WT (C) and M128V (D) expressing neuroblastoma, and the bound Aβ were stained by BCIP/NBT chromogenic stain through biotin and streptavidin–alkaline phosphatase interaction. (E) Flow cytometry analysis for the binding of the Aβ oligomers. Oligomeric biotinylated Aβ42 (0–2500 nM) were added to PrP^C WT and M128V expressing neuroblastoma, and the bound Aβ were stained by FITC-conjugated streptavidin. (F) The binding affinity of the Aβ oligomers (0–2500 nM) to PrP^C WT and M128 V expressing neuroblastoma cells. The mean fluorescence intensity from FITC was plotted against Aβ oligomer concentrations.

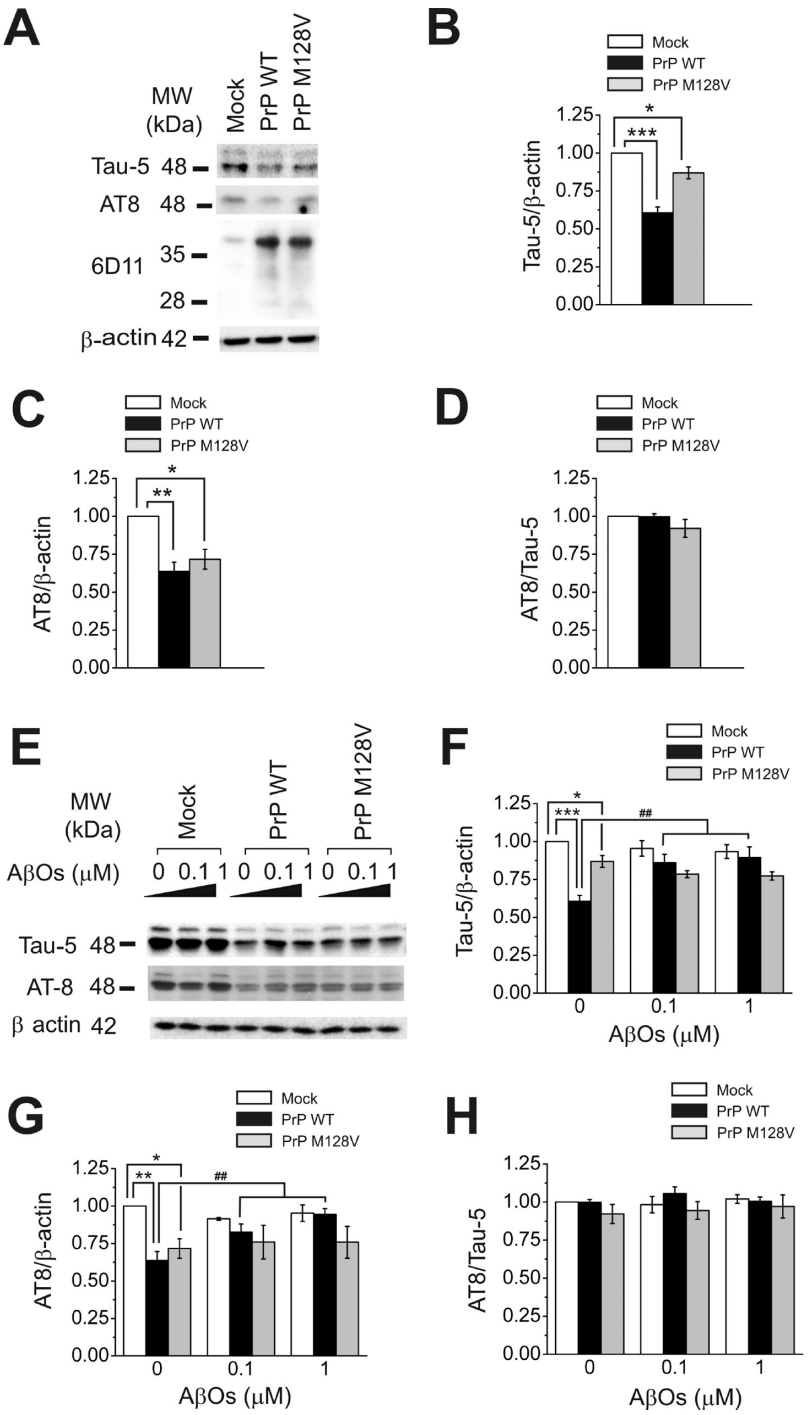


Figure 2. Tau expressions were reduced in PrP^C WT and PrP^C M128V expressing neuroblastoma cells, and the reduction can be rescued by Aβ42 oligomer treatment in PrP^C WT expressing cells. (A) BE(2)-C cells were transfected with empty vectors or vectors containing murine PrP^C WT or M128V cDNAs. The total lysates were immunoblotted with prion antibody 6D11, total tau antibody tau-5, phosphorylated tau antibody AT-8, and β-actin antibody. (B) The quantified and normalized tau-5/β-actin values. Eight replicas were performed. The mock group was used for normalization. All values are presented as mean ± SEM. (C) The quantified and normalized phosphorylated tau (AT8)/β-actin values. Four replicas were performed. The mock group was used for normalization. All values are presented as mean ± SEM. (D) The quantified and normalized phosphorylated tau (AT8)/tau-5 values. Four replicas were performed. The mock group was used for normalization. All values are presented as mean ± SEM. (E) BE(2)-C were transfected with empty vectors or vectors containing murine PrP^C WT or M128V cDNAs for 1 day and treated with different concentrations of Aβ42 oligomers for an additional day. The total lysates were immunoblotted with tau-5, AT-8, and β-actin antibodies. (F) The quantified and normalized values of tau-5/β-actin. Six replicas were performed. The mock group without Aβ oligomer treatment was used for normalization. All values are presented as mean ± SEM. (G) The quantified and normalized values of phosphorylated tau (AT8)/β-actin. Four replicas were performed. The mock group without Aβ oligomer treatment was used for normalization. All values are presented as mean ± SEM. (h) The quantified and normalized phosphorylated tau (AT8)/tau-5 values. Four replicas were performed. The mock group was used for normalization. All values are presented as mean ± SEM. All statistical analyses were performed by Student's t test, (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, and one-way ANOVA, (##) $P < 0.01$.

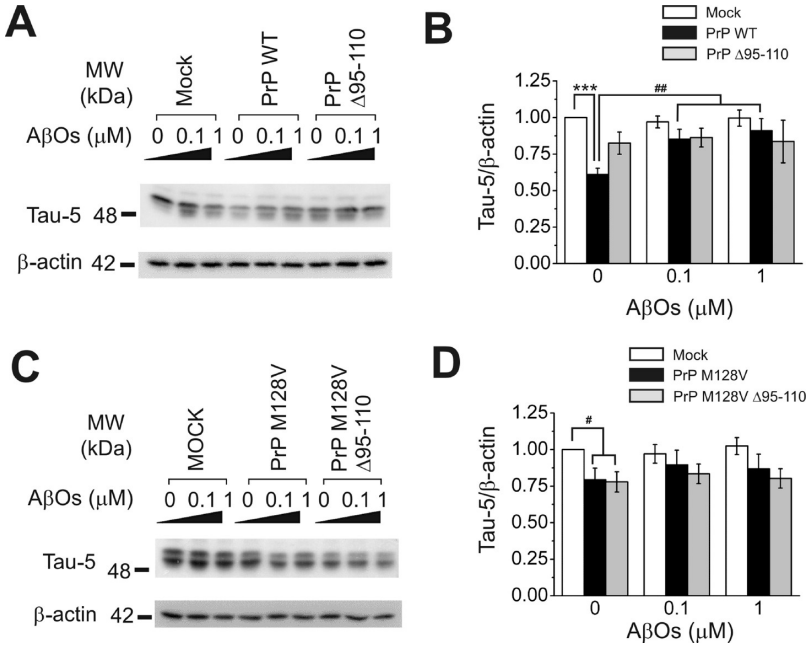


Figure 3. The rescuing effect of Aβ oligomers on tau reduction was diminished in PrP^C Δ95−110 expressing neuroblastoma after Aβ42 oligomer treatment. (A) BE(2)-C cells were transfected with empty vectors or vectors containing murine PrP^C WT or Δ95−110 and treated with different concentrations of Aβ42 oligomers for 1 day. The total lysates were immunoblotted with tau-5 and β-actin antibodies. (B) The quantified and normalized values of tau-5/β-actin from the Western blots. Four replicas were performed. The mock group without Aβ oligomer treatment was used for normalization. All values are presented as mean ± SEM. (C) BE(2)-C cells were transfected with empty vectors or vectors containing murine PrP^C M128V or its Δ95−110 mutant and treated with different concentrations of Aβ42 oligomers for 1 day. The total lysates were immunoblotted with tau-5 and β-actin antibodies. (D) The quantified and normalized values of tau-5/β-actin from the Western blots. Three replicas were performed. The mock group without Aβ oligomer treatment was used for normalization. All values are presented as mean ± SEM. All statistical analyses were performed by Student's t test, (*) $P < 0.05$, (***) $P < 0.001$, and one-way ANOVA, (##) $P < 0.01$.

Moreover, PrP^C and Fyn are required for Aβ oligomer-induced dendritic spine loss, and human AD transgene-induced convulsive seizures do not occur in PrP^C null mice.[14] In contrast, some studies indicate that PrP^C is not required for Aβ oligomer-induced cognitive impairment since the PrP^C expressing and knockout mice have no difference in Aβ oligomer-induced synaptic depression, spine density loss, and deficiency of long-term potentiation.[15] Two intracerebroventricular injections of Aβ oligomers to wild-type or PrP^C null mice have suggested no PrP^C effect on Aβ oligomer-induced memory dysfunction,[10] and modulation of PrP^C in AD transgenic mouse models shows no effect on the impairment of the hippocampal synaptic plasticity.[16] Furthermore, PrP^C deficiency does not ameliorate abnormal neural network activity or cognitive dysfunction in one type of AD model mice.[17] Such controversy may be attributed to only a subset of Aβ oligomers being mediated through PrP^C.[18]

Despite the controversial results from the animal studies,[19] the downstream mechanism of PrP^C binding to Aβ oligomers is not fully elucidated. Here, we would like to further examine the mechanism using the PrP^C overexpressing human neuroblastoma cells in the absence and presence of Aβ oligomers. Our data showed that PrP^C overexpression led to tau reduction via the Fyn pathway and Aβ oligomers modulated PrP^C-induced tau reduction. Our data provide the essential information for the relationship of the three key players in AD, Aβ oligomers, PrP^C, and tau.

RESULTS

PrP^C Expression Induced Tau Reduction That Can Be Relieved by Aβ42 Oligomer Treatment. To understand the downstream mechanism of PrP^C upon Aβ oligomer binding, we first prepared and characterized Aβ42 oligomers.[6] The morphology of Aβ42 oligomers was examined by (TEM) and Western blot (Figure 1). More profound protofibrils and spherical oligomers were observed after oligomer preparation (Figure 1A). The Western blot results showed that after Aβ oligomer preparation, Aβ had more significant amount of higher molecular weight species (>25 kDa), whereas freshly prepared Aβ42 contained mainly monomeric and dimeric species (Figure 1B). The species with a molecular mass around 25 kDa was consistent with the paranuclei found in Aβ42.[20] To confirm the binding affinities of Aβ species to PrP^C, we transfected murine wild-type (WT) PrP^C cDNA to human neuroblastoma BE(2)-C cells and treated them with serially diluted biotinylated Aβ samples ranging from 0 to 2500 nM. The binding was visualized by streptavidin−alkaline phosphatase assay and quantified by flow cytometry. The murine PrP M128V allele corresponding to the human PrP M129V polymorphism that has higher risk of AD was also examined.[8] The results showed Aβ oligomer binding was more significant than the freshly prepared Aβ as expected. The Aβ oligomer binding profiles in WT PrP^C and PrP^C M128V-expressing cells were similar (Figure 1C−F). Our result showing no difference between Aβ oligomer binding to PrP^C and PrP^C M128V is consistent with the previous biophysical study demonstrating identical binding of Aβ oligomers to recombinant human PrP^C and PrP^C M129V by surface plasmon resonance.[9]

Since tau is an essential player in AD, we investigated whether PrP^C affected the tau expression and tau phosphorylation in human neuroblastoma cells. The cells were transfected with either empty vectors (mock group) or vectors

containing WT PrP^C cDNA or PrP^C M128V allele. The cell lysates were examined by Western blotting using anti-PrP^C antibody 6D11, anti-total-tau antibody tau-5, anti-phosphorylated-tau antibody AT-8, and anti-β-actin antibody. Interestingly, we found that total tau protein level was significantly decreased in both PrP^C WT and M128V-expressing cells in comparison to the mock control (Figure 2A,B). The phosphorylated tau was also decreased (Figure 2A,C). The level of tau phosphorylation was not affected when the phosphorylated and total tau ratios were compared (Figure 2D). Next, we examined whether Aβ42 oligomer treatment affected the reduced tau protein induced by PrP^C expression. After treatment with Aβ42 oligomers with different concentrations, 0, 0.1, and 1 μM, for one day, the reduced tau in PrP^C expressing cells was recovered in a dose-dependent manner (Figure 2E,F) and the phosphorylated tau followed the trend (Figure 2E,G). Such increase of total and phosphorylated tau upon Aβ oligomer treatment in PrP^C WT-expressing cells was statistically significant. However, the rescuing effect was not observed in PrP^C M128V-expressing cells after Aβ oligomer treatment, indicating a mechanistic difference resides in PrP^C WT and M128V Aβ signaling. The phosphorylated and total tau ratios were not affected by Aβ oligomer treatment (Figure 2H). The cytotoxicity caused by Aβ oligomers at 1 μM was not profound in MTT assay (see Supporting Information, Figure S1). In addition, we examined the total tau level in the BE(2)-C cells with stable knockdown of endogenous PrP^C. We found the total tau level indeed increased in comparison with the cells infected with the control virus (see Supporting Information, Figure S2). The result confirmed a direct relationship between PrP^C and tau.

To further confirm the Aβ oligomer binding effect, we deleted the major Aβ oligomer binding site on PrP^C, that is, residues 95−110 (PrPΔ95−110), according to previous literature [6] and examined the tau expression level upon Aβ oligomer treatment in PrP^C-expressing cells (Figure 3A,B). In comparison to the mock group, no difference was found in the levels of tau protein in PrP^C Δ95−110-expressing cells upon Aβ oligomer treatment, while the tau level in PrP^C WT expressing cells was increased upon Aβ oligomers treatment. Our results demonstrated that total tau protein can be reduced by PrP^C expression and recovered by Aβ oligomer binding. The same experiments were also performed in M128V PrP^C expressing cells (Figure 3C,D) transfected with PrP M128V and PrP M128V Δ95−110 plasmids. Since no rescuing effect can be seen in PrP M128V expressing cells upon Aβ oligomer treatment, no effect was seen in tau level in the deletion mutant transfected cells.

The tau Reduction Was Attributed to Reduction of tau mRNA Level and the Level Can Be Recovered by Aβ42 Oligomer Treatment in PrP^C Expressing Cells. Next, we examined whether the transcription level of tau gene *MAPT* was affected by PrP^C and Aβ42 oligomers by quantitative real time PCR (qPCR). The results indicated that the tau mRNA levels were decreased to ~61% and ~85% in PrP^C WT and M128V expressing cells, respectively, in comparison to the mock control after one day transfection (Figure 4A). This indicated that the tau protein reduction was attributed to the reduced transcription level of the *MAPT* gene. After Aβ42 oligomer treatment (0, 0.1, 1 μM) for another day, the tau mRNA levels were slightly but significantly increased from ~61% to ~80% in PrP^C WT expressing cells but not in the PrP^C M128V expressing cells (Figure 4B). Furthermore, we

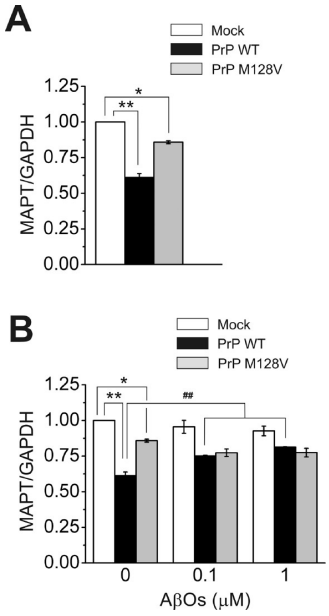


Figure 4. The mRNA level of tau was reduced in PrP^C expressing neuroblastoma cells and can be rescued after Aβ oligomer treatment in PrP^C WT expressing cells. qPCR of *MAPT* in mock group and PrP^C WT or M128V expressing cells with and without Aβ42 oligomer treatment was performed. (A) BE(2)-C cells were transfected with empty vectors or vectors containing murine PrP^C WT or M128V cDNAs. The mRNA levels of tau gene *MAPT* and *GAPDH* were examined. The *MAPT*/*GAPDH* ratios from nine replicas were plotted after normalization with the mock group. All values were presented as mean ± SEM. (B) BE(2)-C cells were transfected with empty vectors or vectors containing murine PrP^C WT or M128 V cDNAs and treated with different concentrations of Aβ42 oligomers for 1 day. The *MAPT*/*GAPDH* ratios after Aβ42 oligomer treatment were plotted. The data from three replicas were normalized to the mock group without Aβ42 oligomer treatment. All values are presented as mean ± SEM. All statistical analyses were performed by Student's *t* test, (*) $P < 0.05$, (**) $P < 0.01$, and one-way ANOVA, (##) $P < 0.01$.

asked whether the reduction of tau protein level can be attributed to alteration of proteasome activity. We employed proteasome activity measurement in PrP^C and PrP^C M128V expressing cells and found that there was no significant difference in the proteasome activity, indicating that the transcriptional regulation is the predominant factor in PrP^C-induced tau reduction (data not shown). Thus, our data suggested that the effect of PrP^C on tau protein was related to the transcriptional regulation of *MAPT* and Aβ42 oligomer binding to PrP^C WT but not PrP^C M128V is capable of regulating the transcription.

Fyn Pathway Inhibitors Alleviated the PrP^C-Induced Tau Reduction. To examine the possible signaling pathways involved in the PrP^C induced tau reduction, we examined the Fyn pathway since Fyn, a Src family kinase, is a downstream kinase of PrP^C that can form a complex with PrP^C and has been reported to respond to Aβ oligomers.[14,21] We treated both the mock group and PrP^C expressing cells with the Src kinase family inhibitor PP2 at 1 μM and its negative control PP3 (Figure 5A,B). The reduction of tau in the PP3 treated groups remained reduced. However, the reduced tau level was significantly recovered by PP2 treatment in the PrP^C expressing cells. The dose-dependent effect of PP2 to rescue the tau expression level was also demonstrated (Figure 5C,D). The potent inhibition of Fyn kinase activity by PP2 treatment was

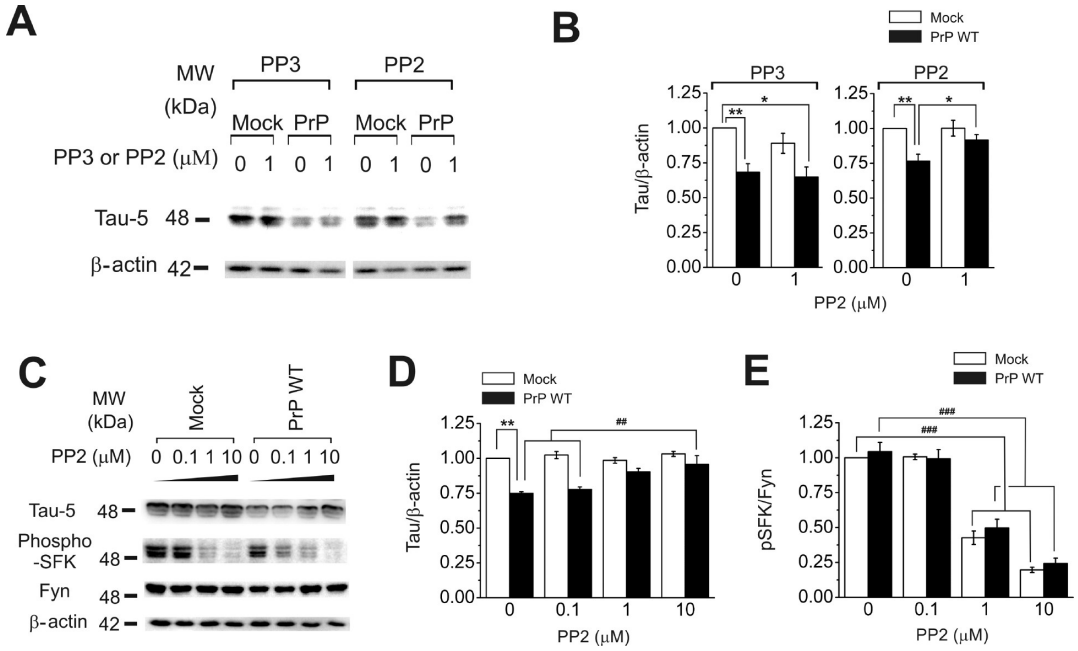


Figure 5. The PrP^C induced tau reduction can be rescued by Fyn kinase inhibitor. (A) BE(2)-C cells were transfected with the empty vectors or vectors containing PrP^C with and without 1 μM Src kinase inhibitor PP2 or non-Fyn kinase inhibitor PP3 as a negative control. The total lysates were immunoblotted with the total tau antibody tau-5 and β-actin antibody. (B) The quantified and normalized values of tau-5/β-actin with PP2 or PP3 treatments. More than four replicas were performed. The data were normalized to the mock group without treatment. All values are presented as mean ± SEM. The statistical analyses were performed by Student's t test, (*) $P < 0.05$, (**) $P < 0.01$. (C) The tau, phosphorylated Fyn, and total Fyn levels under dose-dependent PP2 treatment in mock group and PrP^C WT expressing cells. Different PP2 concentrations ranging from 0 to 10 μM were employed. (D,E) The quantified and normalized values of tau-5/β-actin (D) and phosphorylated Fyn/Fyn (E) with dose-dependent PP2 treatment. Four replicas were performed. The data were normalized to the mock group without treatment. All values are presented as mean ± SEM. The statistical analyses were performed by Student's t test, (**) $P < 0.01$, and one-way ANOVA, (##) $P < 0.01$; (###) $P < 0.001$.

further confirmed by Western blotting probed by anti-phospho-SFK (Src family kinase) and total Fyn antibodies (Figure 5C,E). Above 1 μM PP2, the phosphorylated Fyn was significantly reduced in comparison to the total Fyn level. We further used an inhibitor of MEK, U0126, to examine the Fyn signaling pathway. MEK is a downstream regulator in the Fyn pathway. U0126 treatment from 0.1 to 10 μM also reversed the tau reduction observed in PrP^C WT-expressing cells (Figure 6). The fact that Fyn kinase and MEK inhibitors both reversed the PrP^C-induced tau reduction indicated that the tau reduction was regulated via Fyn pathway.

Aβ Oligomer Treatment Modulated Fyn Kinase Activity. To understand whether Fyn kinase was involved in the Aβ oligomer treatment, we examined the phosphorylated Fyn and total Fyn levels in mock and PrP^C transfected cells after 2 h and 1 day Aβ oligomer treatments (Figure 7). We found after 2 h of Aβ oligomer treatment, phosphorylated Fyn level increased in a dose-dependent manner with Aβ oligomer concentration where the total Fyn level was constant. The phenomenon was consistent with the previous literature stating Aβ oligomer binding to PrP^C complex results in Fyn activation.[14] However, after 1 day of Aβ oligomer treatment, the ratio of phosphorylated Fyn and total Fyn decreased. The inactivation of Fyn after 1 day of Aβ oligomer treatment was consistent with the observed tau rescuing effect seen under Fyn kinase inhibitor and Aβ oligomer treatments. The phenomenon was further confirmed by immunoprecipitation (IP) with anti-Fyn antibody (data not shown). Therefore, we confirmed that after 1 day of Aβ oligomer treatment in the PrP^C transfected cells, Fyn kinase was inactivated. Our results further demonstrated that Fyn pathway is the main signaling

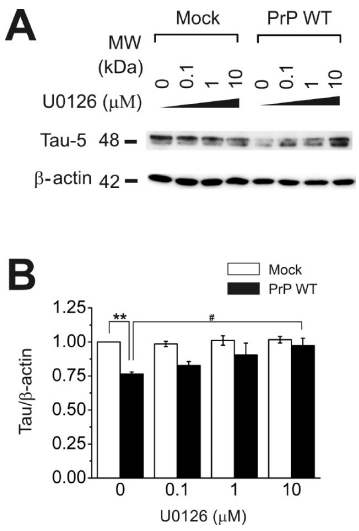


Figure 6. The PrP^C induced tau reduction can be rescued by MEK inhibitor. (A) BE(2)-C cells were transfected with the empty vectors or vectors containing PrP^C with and without different concentrations of MEK inhibitor U0126. The final U0126 concentrations were 0, 0.1, 1, and 10 μM. The total lysates were immunoblotted with the total tau antibody tau-5 and β-actin antibody. (B) The quantified and normalized values of tau-5/β-actin with dose-dependent U0126 treatment. Three replicas were performed. The data were normalized to the mock group without treatment. All values are presented as mean ± SEM. The statistical analyses were performed by Student's t test, (**) $P < 0.01$, and one-way ANOVA, (#) $P < 0.05$.

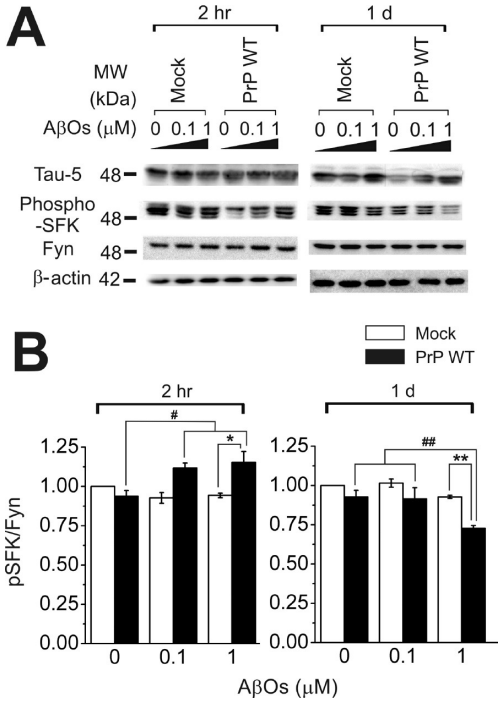


Figure 7. Aβ oligomer treatment modulated Fyn kinase activity. (A) BE(2)-C cells were transfected with the empty vectors or vectors containing PrP^C WT with and without Aβ42 oligomer treatment (0, 0.1, 1 μM) for 2 h or 1 day. The total lysates were immunoblotted with tau-5, phospho-SFK, Fyn, and β-actin antibodies. (B) The quantified and normalized values of phosphorylated Fyn/Fyn with dose-dependent Aβ oligomer treatment. More than three replicas were performed. The data were normalized to the mock group without treatment. All values are presented as mean $\pm$ SEM. The statistical analyses were performed by Student's t test, (*) $P < 0.05$; (**) $P < 0.01$, and one-way ANOVA, (#) $P < 0.05$; (##) $P < 0.01$.

responsible for the tau transcriptional regulation mediated by PrP^C and Aβ oligomers.

■ DISCUSSION

Aβ aggregation is crucial to the etiology of AD in which the prefibrillar oligomers are considered the actual toxic entities. Aβ has been suggested to trigger tau-mediated neurotoxicity by enhancing tau hyperphosphorylation.[3] In our study, we observed consistently a reduction of total tau protein level in the PrP^C expressing cells where the changes in tau hyperphosphorylation followed the trend in total tau. Aβ oligomer binding alleviated the reduction of tau in PrP^C but not PrP^C M128V expressing cells. Our qPCR data showed coordinate changes in tau transcriptional level with the protein expression level. Since the PrP^C WT and PrP^C M128V expressing cells both showed tau reduction in protein and mRNA levels albeit the M128V polymorphism was less potent in tau reduction, we suggested that the M128V polymorphic effect did not alter the tau reduction qualitatively but quantitatively.

Several signaling and adhesion molecules have been reported to be PrP^C binding partners. For example, PrP^C can interact with neural cell adhesion molecule (NCAM) and recruit it to lipid rafts to activate Fyn kinase,[22] and stimulation of PrP^C signaling in PC12 cells by PrP^C antibody results in phosphorylation of caveolin-1, which inactivates Fyn that stops the downstream signaling.[23] In fact, PrP^C/caveolin-1/Fyn complex has been found in AD patients and transgenic mice.[21] Besides, cross-linked Aβ and PrP^C complex were found in lipid rafts after activation of synaptic cytoplasmic phospholipase A2 (cPLA2) by Aβ oligomers where lesser effect was found in the neurons of PrP^C null mice.[11] The accumulation of Aβ oligomers to lipid rafts were found to be Fyn-dependent.[24] Interestingly, Aβ oligomers have been shown to induce cell surface retention of PrP^C in both cell lines and mouse hippocampal neurons[25] suggesting Aβ oligomer interaction with PrP^C may regulate PrP^C endocytosis. Here, our results showed that Aβ oligomers reversed the PrP^C-induced tau reduction. We suspect that the result is attributed from Aβ-induced PrP^C surface retention that interfered with the caveolae-mediated PrP^C endocytosis and Fyn activation. Further intensive studies are needed to elucidate the mechanism. It is intriguing that Aβ oligomers were able to bind to the PrP^C M128V polymorphic allele but unable to rescue the tau reduction. The possible mechanisms could be related to PrP^C conformational changes in the M128V allele or the possible discrepancy between PrP^C and PrP^C M128V cell surface retention induced by Aβ oligomers. Although PrP^C has been reported to be located in nuclear lamina of neuronal cells in the mice brain tissues and interact with histone H3, which suggests that PrP^C may be involved in transcriptional regulation in the nucleus,[26] our preliminary data and most other studies[25,27] showed PrP^C did not go into nucleus indicating that a direct influence of PrP^C in the transcriptional regulation is unlikely.

Fyn kinase is an emerging therapeutic target for AD.[28] Recently, Fyn activation has been reported in AD brain tissue and AD mice indicating the importance of Fyn kinase in the pathogenic mechanisms of AD.[14,21] Fyn can be abnormally sorted to dendrites via tau and Fyn interaction.[29] It is also involved in synaptic plasticity that contributes to learning and memory and regulates NMRAR and AMPAR activity, trafficking, and degradation. Our data showed that treatment of Fyn pathway inhibitors such as Fyn kinase or MEK inhibitors can reverse the tau reduction effect induced by PrP^C. Although MAPK signaling is implicated downstream of Aβ−PrP^C−Fyn, how MAPK signaling is linked to tau gene transcription is currently unknown. In the tau promoter region, there are several transcription factor binding sites including Sp1, GCP, and AP-2.[30] Although Sp1 is shown to be a downstream regulator of MAPK p38 in non-neuronal systems,[31] whether PrP^C-induced *MAPT* reduction is simply related to Sp1 or other possible gene suppressions such as chromatin modification[32] requires further intensive investigation. Besides using Fyn pathway inhibitors to study the signaling, we had also examined the phosphorylated Fyn level after Aβ oligomer treatment and found that the Fyn kinase was activated after 2 h of Aβ oligomer treatment. However, after 1 day of treatment, Fyn activation was decreased. The Fyn inactivation was consistent with our results suggesting Aβ oligomer regulated PrP^C-induced tau reduction via the Fyn pathway. The mechanism is illustrated in Figure 8. Overall, our data demonstrated for the first time that tau can be reduced via the Fyn pathway in a PrP^C-dependent manner and the event can be regulated by Aβ oligomer treatment. Since tau has been demonstrated to be essential to Aβ-induced neuronal dysfunction,[5] the PrP^C-induced tau reduction may reflect the physiological function of PrP^C and be helpful for future therapeutic development for AD.

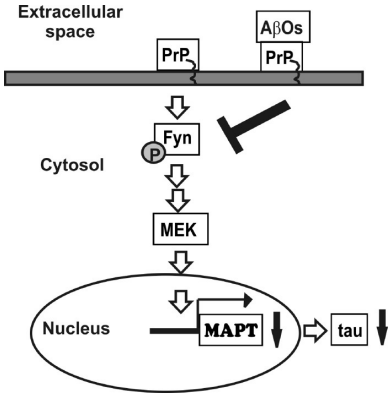


Figure 8. Illustration of Aβ−PrP^C−tau signaling via Fyn pathway. Molecules involved in the Aβ−PrP^C−tau signaling and the relative subcellular localizations are indicated.

■ MATERIALS AND METHODS

Preparation of Aβ42 Oligomers. Synthetic WT Aβ42 peptide was synthesized by the Genomic Research Center peptide facility using Fmoc (*N*-(9-fluorenyl) methoxycarbonyl) chemistry and purified by reversed phase high-performance liquid chromatography. The molecular mass was identified by MALDI-TOF mass spectrometry (UltraFlex II; Bruker BioSciences). The biotinylated Aβ42 used for the cellular binding study was purchased from Biopeptide (San Diego, CA, USA). To prepare the Aβ42 oligomers, the lyophilized peptide was freshly treated with 100% hexafluoroisopropanol (HFIP) for 1 h to eliminate pre-existing Aβ42 aggregates. A thin film of Aβ42 was formed after lyophilization. The thin film was then dissolved by DMSO at a concentration of 5 mM and sonicated for 5 min. Finally, oligomer preparation was initiated by adding the dissolved peptides to DMEM/F12 (GIBCO, Invitrogen) at a final peptide concentration of 100 μM, and the sample was incubated without agitation at 4 °C for 24 h.[33] The Aβ oligomer stocks were freshly prepared and diluted to the working concentrations presented in Aβ monomer equivalent concentration as indicated for each experiment.

Transmission Electron Microscopy (TEM). Freshly prepared Aβ42 or Aβ42 oligomers were placed on glow-discharged, 400-mesh Formvar carbon-coated copper grids (EMS Ins.) for 5 min. Then, the grids were rinsed with ddH_2O and negatively stained with 2% uranyl acetate for 5 min. The morphologies of Aβ oligomers were observed by a Tecnai G2 Spirit TWIN TEM (FEI) with an accelerating voltage of 75 kV.

Cellular Binding of Aβ42 Oligomers. The cells were seeded at 8×10^4 cells per well in 96 well plates. The cells were transfected with pCMV-SPORT6-PrP WT or M128V plasmids with Lipofectamine 2000 (Invitrogen) for 2 days. Then, the cells were treated with serially diluted biotinylated Aβ42 oligomers for 2 h at 37 °C with the final concentrations ranging from 0 to 2500 nM. The cells were washed with media and fixed in 3.7% formaldehyde. After fixation, the cells were washed with 1× PBS, incubated at 65 °C for 2 h, and then blocked with PBS containing 3% goat serum and 0.1% Triton X-100. The bound Aβ42 was detected by adding alkaline phosphatase-conjugated streptavidin (1:1000 dilution, Perki-nElmer) and its substrate, 50 μL of BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) (Perki-nElmer). The reactions were stopped after color changed at room temperature. The level of Aβ oligomer binding was also measured by flow cytometry. The cells were seeded in a 12 well plate at a density of 2×10^5 cells/well. The cells were transfected with PrP WT or M128V plasmids and then treated with biotinlyated Aβ42 oligomers with indicated concentrations for 5 min. The cells were washed with ice-cold 1× PBS, suspended, and incubated with FITC conjugated streptavidin (1:500 dilution, BD Biosciences) on ice for 1 h. After wash, the cells were fixed with 1% formaldehyde in PBS for 15 min. Finally, the cells were washed with 1× PBS, and the FITC fluorescence was quantified by a FACSCanto flow cytometer (BD Biosciences).

Plasmids. The pCMV-SPORT6-PrP^C plasmid encoding murine WT PrP^C cDNA was purchased from Thermo Scientific (Open Biosystem) and verified by DNA sequencing. The M128V and the amino acids 95−110 deletion mutants were performed using site-directed mutagenesis with PfuUltra II Fusion HS DNA Polymerase (Stratagene). The primers used for M128V were the forward primer with A382G mutation 5′-CCT TGG TGG CTA CGT GCT GGG GAG CG-3′ and the reverse primer 5′-CGC TCC CCA GCA CGT AGC CAC CAA GG-3′, and those for the deletion mutant were the forward primer 5′-GTG GCA GGG GCT GCG GCA-3′ and the reverse primer 5′-GGT ACC CCC TCC TTG GCC C-3′.

Cell Culture and Transfection. Human neuroblastoma BE(2)-C cells were grown in RPMI media (GIBCO, Invitrogen) containing 10% fetal bovine serum (GIBCO, Invitrogen) at 37 °C under 5% CO_2. All constructs were transfected into cells using transfection reagent Lipofectamine 2000 (Invitrogen) for 1 day. Then, the transfected cells were treated with Aβ oligomers (0, 0.1, and 1 μM) for an additional day. The cells were harvested in PBS and subjected to Western blotting.

Western Blotting. Cells were lysed in RIPA lysis buffer containing 50 mM Tris, pH 7.0, 150 mM NaCl, 0.25% sodium deoxycholate, 1% NP-40, 1 mM EDTA, 1× protease inhibitor cocktail (Merck Millipore), and 1× phosphatase inhibitor cocktail (Roach). Lysates were centrifuged at 17 000 × *g* for 30 min at 4 °C. The total protein was quantified by BCA protein assay kit (Thermo, Pierce), and 20 μg of protein was loaded onto 16% Tris-tricine gel or 12% Tris-glycine SDS-PAGE and analyzed by Western blotting. Antibodies used for Western blots were as follows: anti-Aβ, 6E10 (1:4000, Covance), anti-total tau, tau5 (Invitrogen), anti-phosphorylated tau, AT-8 (Thermo Scientific), anti-PrP^C antibodies 6D11 (Covance), anti-β-actin (1:10,000, Gentex), anti-phospho-Src (Tyr416) kinase antibody #2101 (phospho-SFK, Cell signaling Technology), and anti-Fyn antibody #4023 (Cell signaling Technology). The epitopes of anti-PrP^C antibodies 6D11, 7D9, and C-term are prion a.a. 93−109, a.a. 23−237, and C-terminal prion, respectively. All primary antibodies were performed at 1:2000 dilution unless otherwise indicated. The secondary antibodies were HRP-conjugated goat anti-mouse or goat anti-rabbit IgG (1:5000). The densitometry analyses were performed by Image J (NIH, USA).

Quantitative Real-Time PCR (qPCR). Total RNA was prepared with TRIzol Reagent (Invitrogen), and cDNA synthesis was carried out with oligo dT primers by using SuperScript III Fist-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. qPCR was performed with LightCycler 480 real-time PCR system (Roche) using KAPA SYBR FAST qPCR kit (Kapa Biosystems) with specific primers. The thermocycling conditions used were as follows: an initial step of 10 min at 95 °C, 40 cycles of a 15 s denaturation at 95 °C, 20 s annealing at 60

°C, and 1 s extension at 72 °C. The cycle threshold, Ct, of each sample was generated with the default setting. The *MAPT* expression level of each sample was normalized to the expression level of *GAPDH* in the same sample by the following formula: $MAPT/GAPDH = 2^{-(\mathrm{Ct}(MAPT)-\mathrm{Ct}(GAPDH))}$. The *MAPT*/*GAPDH* ratio of mock transfected cells was set to 1.0, and the values of all others were recalculated accordingly. Sequences of primers were as follows: *MAPT*, 5′-AAG TCG CCG TCT TCC GCC AAG-3′ and 5′-AAG TCG CCG TCT TCC GCC AAG-3′; *GAPDH,* 5′-TCT TTT GCG TCG CCA GCC GAG-3′ and 5′-AAG TCC CGT TCT CAG CCT TGA CGG T-3′.

Fyn Pathway Inhibitor Treatments. Human neuroblastoma BE(2)-C cells were grown and transfected following previous descriptions. The one day transfected cells were treated with Fyn pathway inhibitors for an additional day. The Fyn pathway inhibitor stocks were dissolved in 100% DMSO. The final DMSO concentrations in the media were less than 0.1%. The corresponding buffers were treated in the no treatment groups. The Src family kinase inhibitor PP2 (Millipore) and its negative control PP3 (Millipore) were treated as indicated. The final MEK inhibitor U0126 (Cell Signaling Technology) concentrations were 0, 0.1, 1, and 10 μM.

Statistical Analysis. The quantitative data were presented with the averaged mean values and the mean of standard deviation (SEM) from at least three separate experiments. Most data were compared with the mock control as indicated, and the statistical analyses were performed by the paired Student's *t* test. The actual *P* values are presented. To compare more than two groups, the statistical analyses were performed by the one-way ANOVA with Tukey's multiple comparison test.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Methods and results on cytotoxicity of Aβ oligomers and PrP^C knockdown. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Mailing address: Genomics Research Center, Academia Sinica, 128, Academia Rd, Section 2, Nankang District, Taipei 115, Taiwan. E-mail: yrchen@gate.sinica.edu.tw. Telephone:+886-2-2787-1275. Fax: +886-2-2789-8771.

Author Contributions

R.J.C. designed and performed major experiments. W.W.C. performed flow cytometry, toxicity, and other experiments. Y.C.L. assisted on cellular experiments. P.L.C. provided critical suggestions. Y.R.C. designed and directed the research direction. R.J.C. and Y.R.C. wrote the paper.

Funding

This work was supported by Academia Sinica, Taiwan (Grant AS-100-TP2-B01), and the National Science Council, Taiwan (Grants NSC 98-2320-B-001-020-MY3 and NSC 101-2320-B-001-035).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. Alice Yu, Genomics Research Center, Academia Sinica, for sharing the BE(2)-C cells and the Genomics Research Center peptide synthesis facility for synthesizing Aβ42. We thank Profs. Yung-Feng Liao, Inst. of Cellular and Organismic Biology, Academia Sinica, and Pang-Hsien Tu, Inst. of Biomedical Science, Academia Sinica, for critical suggestions.

■ REFERENCES

(1) (a) Lesne, S., Koh, M. T., Kotilinek, L., Kayed, R., Glabe, C. G., Yang, A., Gallagher, M., and Ashe, K. H. (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature 440* (7082), 352−35. (b) Shankar, G. M., Li, S., Mehta, T. H., Garcia-Munoz, A., Shepardson, N. E., Smith, I., Brett, F. M., Farrell, M. A., Rowan, M. J., Lemere, C. A., Regan, C. M., Walsh, D. M., Sabatini, B. L., and Selkoe, D. J. (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med. 14* (8), 837−842.

(2) Haass, C., and Selkoe, D. J. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat. Rev. Mol. Cell Biol. 8* (2), 101−112.

(3) Morris, M., Maeda, S., Vossel, K., and Mucke, L. (2011) The many faces of tau. *Neuron 70* (3), 410−426.

(4) Avila, J., Lucas, J. J., Perez, M., and Hernandez, F. (2004) Role of tau protein in both physiological and pathological conditions. *Physiol. Rev. 84* (2), 361−384.

(5) (a) Roberson, E. D., Scearce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., Gerstein, H., Yu, G. Q., and Mucke, L. (2007) Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science 316* (5825), 750−754. (b) Vossel, K. A., Zhang, K., Brodbeck, J., Daub, A. C., Sharma, P., Finkbeiner, S., Cui, B., and Mucke, L. (2010) Tau reduction prevents Abeta-induced defects in axonal transport. *Science 330* (6001), 198.

(6) Lauren, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W., and Strittmatter, S. M. (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature 457* (7233), 1128−1132.

(7) (a) Takahashi, R. H., Tobiume, M., Sato, Y., Sata, T., Gouras, G. K., and Takahashi, H. (2011) Accumulation of cellular prion protein within dystrophic neurites of amyloid plaques in the Alzheimer's disease brain. *Neuropathology 31* (3), 208−214. (b) Ferrer, I., Blanco, R., Carmona, M., Puig, B., Ribera, R., Rey, M. J., and Ribalta, T. (2001) Prion protein expression in senile plaques in Alzheimer's disease. *Acta Neuropathol. 101* (1), 49−56.

(8) (a) Dermaut, B., Croes, E. A., Rademakers, R., Van den Broeck, M., Cruts, M., Hofman, A., van Duijn, C. M., and Van Broeckhoven, C. (2003) PRNP Val129 homozygosity increases risk for early-onset Alzheimer's disease. *Ann. Neurol. 53* (3), 409−412. (b) Riemenschneider, M., Klopp, N., Xiang, W., Wagenpfeil, S., Vollmert, C., Muller, U., Forstl, H., Illig, T., Kretzschmar, H., and Kurz, A. (2004) Prion protein codon 129 polymorphism and risk of Alzheimer disease. *Neurology 63* (2), 364−366.

(9) Chen, S., Yadav, S. P., and Surewicz, W. K. (2010) Interaction between human prion protein and amyloid-beta (Abeta) oligomers: Role of N-terminal residues. *J. Biol. Chem. 285* (34), 26377−26383.

(10) Balducci, C., Beeg, M., Stravalaci, M., Bastone, A., Sclip, A., Biasini, E., Tapella, L., Colombo, L., Manzoni, C., Borsello, T., Chiesa, R., Gobbi, M., Salmona, M., and Forloni, G. (2010) Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein. *Proc. Natl. Acad. Sci. U. S. A. 107* (5), 2295−2300.

(11) Bate, C., and Williams, A. (2011) Amyloid-{beta}-induced synapse damage is mediated via cross-linkage of the cellular prion protein. *J. Biol. Chem. 286* (44), 37955−37963.

(12) (a) Chung, E., Ji, Y., Sun, Y., Kascsak, R. J., Kascsak, R. B., Mehta, P. D., Strittmatter, S. M., and Wisniewski, T. (2010) Anti-PrPC monoclonal antibody infusion as a novel treatment for cognitive deficits in an Alzheimer's disease model mouse. *BMC Neurosci. 11*, No. 130. (b) Barry, A. E., Klyubin, I., Mc Donald, J. M., Mably, A. J., Farrell, M. A., Scott, M., Walsh, D. M., and Rowan, M. J. (2011) Alzheimer's disease brain-derived amyloid-beta-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. *J.*

Neurosci. 31 (20), 7259−7263. (c) Freir, D. B., Nicoll, A. J., Klyubin, I., Panico, S., Mc Donald, J. M., Risse, E., Asante, E. A., Farrow, M. A., Sessions, R. B., Saibil, H. R., Clarke, A. R., Rowan, M. J., Walsh, D. M., and Collinge, J. (2011) Interaction between prion protein and toxic amyloid beta assemblies can be therapeutically targeted at multiple sites. *Nat. Commun. 2*, 336.

(13) Resenberger, U. K., Harmeier, A., Woerner, A. C., Goodman, J. L., Muller, V., Krishnan, R., Vabulas, R. M., Kretzschmar, H. A., Lindquist, S., Hartl, F. U., Multhaup, G., Winklhofer, K. F., and Tatzelt, J. (2011) The cellular prion protein mediates neurotoxic signalling of beta-sheet-rich conformers independent of prion replication. *EMBO J. 30* (10), 2057−2070.

(14) Um, J. W., Nygaard, H. B., Heiss, J. K., Kostylev, M. A., Stagi, M., Vortmeyer, A., Wisniewski, T., Gunther, E. C., and Strittmatter, S. M. (2012) Alzheimer amyloid-beta oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nat. Neurosci. 15* (9), 1227−1235.

(15) Kessels, H. W., Nguyen, L. N., Nabavi, S., and Malinow, R. (2010) The prion protein as a receptor for amyloid-beta. *Nature 466* (7308), E3−E4. () E4−E5.

(16) Calella, A. M., Farinelli, M., Nuvolone, M., Mirante, O., Moos, R., Falsig, J., Mansuy, I. M., and Aguzzi, A. (2010) Prion protein and Abeta-related synaptic toxicity impairment. *EMBO Mol. Med. 2* (8), 306−314.

(17) Cisse, M., Sanchez, P. E., Kim, D. H., Ho, K., Yu, G. Q., and Mucke, L. (2011) Ablation of cellular prion protein does not ameliorate abnormal neural network activity or cognitive dysfunction in the J20 line of human amyloid precursor protein transgenic mice. *J. Neurosci. 31* (29), 10427−10431.

(18) Larson, M. E., and Lesne, S. E. (2012) Soluble Abeta oligomer production and toxicity. *J. Neurochem. 120* (Suppl 1), 125−139.

(19) Benilova, I., and De Strooper, B. (2010) Prion protein in Alzheimer's pathogenesis: a hot and controversial issue. *EMBO Mol. Med. 2* (8), 289−290.

(20) Bitan, G., Kirkitadze, M. D., Lomakin, A., Vollers, S. S., Benedek, G. B., and Teplow, D. B. (2003) Amyloid beta-protein (Abeta) assembly: Abeta 40 and Abeta 42 oligomerize through distinct pathways. *Proc. Natl. Acad. Sci. U. S. A. 100* (1), 330−335.

(21) Larson, M., Sherman, M. A., Amar, F., Nuvolone, M., Schneider, J. A., Bennett, D. A., Aguzzi, A., and Lesne, S. E. (2012) The complex PrPc-Fyn couples human oligomeric Abeta with pathological tau changes in Alzheimer's disease. *J. Neurosci. 32* (47), 16857−16871.

(22) Santuccione, A., Sytnyk, V., Leshchyns'ka, I., and Schachner, M. (2005) Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth. *J. Cell Biol. 169* (2), 341−354.

(23) Pantera, B., Bini, C., Cirri, P., Paoli, P., Camici, G., Manao, G., and Caselli, A. (2009) PrPc activation induces neurite outgrowth and differentiation in PC12 cells: Role for caveolin-1 in the signal transduction pathway. *J. Neurochem. 110* (1), 194−207.

(24) Williamson, R., Usardi, A., Hanger, D. P., and Anderton, B. H. (2008) Membrane-bound beta-amyloid oligomers are recruited into lipid rafts by a fyn-dependent mechanism. *FASEB J. 22* (5), 1552−1559.

(25) Caetano, F. A., Beraldo, F. H., Hajj, G. N., Guimaraes, A. L., Jurgensen, S., Wasilewska-Sampaio, A. P., Hirata, P. H., Souza, I., Machado, C. F., Wong, D. Y., De Felice, F. G., Ferreira, S. T., Prado, V. F., Rylett, R. J., Martins, V. R., and Prado, M. A. (2011) Amyloid-beta oligomers increase the localization of prion protein at the cell surface. *J. Neurochem. 117* (3), 538−553.

(26) Strom, A., Wang, G. S., Picketts, D. J., Reimer, R., Stuke, A. W., and Scott, F. W. (2011) Cellular prion protein localizes to the nucleus of endocrine and neuronal cells and interacts with structural chromatin components. *Eur J Cell Biol 90* (5), 414−419.

(27) Rushworth, J. V., Griffiths, H. H., Watt, N. T., and Hooper, N. M. (2013) Prion protein-mediated toxicity of amyloid-beta oligomers requires lipid rafts and the transmembrane LRP1. *J. Biol. Chem. 288* (13), 8935−8951.

(28) Yang, K., Belrose, J., Trepanier, C. H., Lei, G., Jackson, M. F., and MacDonald, J. F. (2011) Fyn, a potential target for Alzheimer's disease. *J. Alzheimer's Dis. 27* (2), 243−252.

(29) Ittner, L. M., Ke, Y. D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wolfing, H., Chieng, B. C., Christie, M. J., Napier, I. A., Eckert, A., Staufenbiel, M., Hardeman, E., and Gotz, J. (2010) Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell 142* (3), 387−397.

(30) (a) Andreadis, A., Wagner, B. K., Broderick, J. A., and Kosik, K. S. (1996) A tau promoter region without neuronal specificity. *J. Neurochem. 66* (6), 2257−2263. (b) Heicklen-Klein, A., and Ginzburg, I. (2000) Tau promoter confers neuronal specificity and binds Sp1 and AP-2. *J. Neurochem. 75* (4), 1408−1418.

(31) (a) Xu, K., and Shu, H. K. (2007) EGFR activation results in enhanced cyclooxygenase-2 expression through p38 mitogen-activated protein kinase-dependent activation of the Sp1/Sp3 transcription factors in human gliomas. *Cancer Res. 67* (13), 6121−6129. (b) D'Addario, M., Arora, P. D., and McCulloch, C. A. (2006) Role of p38 in stress activation of Sp1. *Gene 379*, 51−61.

(32) Yang, S. H., Sharrocks, A. D., and Whitmarsh, A. J. (2013) MAP kinase signalling cascades and transcriptional regulation. *Gene 513* (1), 1−13.

(33) Dahlgren, K. N., Manelli, A. M., Stine, W. B., Jr., Baker, L. K., Krafft, G. A., and LaDu, M. J. (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J. Biol. Chem. 277* (35), 32046−32053.