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Novel therapeutic strategies for neurodegenerative disease

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Abstract

The activity of protein phosphatase 2A (PP2A) is compromised and believed to be the cause of the abnormal hyperphosphorylation of tau in Alzheimer's disease (AD) brain. Activity of PP2A is regulated by two endogeneous inhibitor proteins, called as I_1^{PP2A} and I_2^{PP2A}. Previously, we reported that: (i) I_1^{PP2A} and I_2^{PP2A} are upregulated with cleavage of I_2^{PP2A} holoprotein and translocation of its amino terminal fragment from the nucleus to the cytoplasm in neuronal cells in AD brains; and (ii) translocated I_2^{PP2A} colocalized not only with the PP2A catalytic subunit, but also with phosphorylated tau in neuronal cytoplasm. Furthermore, according to preliminary data, the cleavage site of I_2^{PP2A} is located between amino acids 175 and 176 of the I_2^{PP2A} sequence. Because the sequence from amino acids 168 to 181 on I_2^{PP2A} presumably functions as a nuclear localization signal (NLS), inhibition of break down of the NLS in I_2^{PP2A} is expected to be a novel therapeutic target for the treatment of Alzheimer's disease.

Keywords

Alzheimer's disease; I_2^{PP2A}; neurofibrillary tangle; protein phosphatase 2A; tau

INTRODUCTION

In recent years, there has been a rapid increase in the elderly population worldwide; consequently, the number of patients with dementia has also increased. At present there are approximately 1 700 000 patients with dementia in Japan, and it is estimated that this figure will increase to 2 500 000 in 2015 and to 3 230 000 in 2025.[1] Thus, the aging society is one of the most important issues not only from a social viewpoint, but also from a psychogeriatric medical point of view. Behavioral and psychiatric symptoms of dementia (BPSD) are one of the major issues for care and therapy,[2,3] and therapeutic approaches to the treatment of BPSD have been developed.[4–8] Although treatment of BPSD is important from a social point of view, therapy for the pathological process of dementia, namely neurodegenerative disease, is essential from the medical point of view.

Alzheimer's disease (AD) is a major neurodegenerative disease and the underlying pathological mechanisms are under intense investigation worldwide. Alzheimer's disease is

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characterized by progressive neurodegeneration and there are two types of abnormal deposits that are observed pathologically in AD brains, namely neurofibrillary tangles (NFT) and extracellualr amyloid plaques, composed of hyperphosphorylated tau forming paired helical filaments (PHF) and amyloid β (Aβ) peptide.[9,10] As pathological components of AD, Aβ and tau have been investigated in considerable and the results of this research applied for the early diagnosis and therapy of AD.[11–14]

At present, the main therapeutic target in AD is Aβ and several therapeutic approaches have been developed accordingly, including β/γ secretase inhibitor(s)/modulator(s) and Aβ vaccines. However, alternative or supplemental therapies are required.

In the present review, one of the possible therapeutic targets for neurofibrillary degeneration, especially targeting the hyperphosphorylation of tau, in AD brain is discussed.

FUNCTION OF TAU PROTEIN AND ITS COMPROMISE IN AD BRAIN

Tau protein is one of the major microtubule-associated proteins that is mainly localized in neuronal axons, as well as in neuronal cell bodies, dendrites, and non-neuronal cells, such as astrocytes and oligodendrocytes. The major biological function of tau is to promote microtubule assembly and maintain the stability of the previously formed microtubules, which are essential for the axonal transport of the neurons.

When tau is hyperphosphorylated, several crucial events occur, including: (i) loss of the ability to promote microtubule assembly; (ii) binding with and sequestration of normal unphosphorylated tau from microtubules; and (iii) promotion of the self-assembly of normal unphosphorylated tau into PHF.

REGULATION OF TAU PHOSPHORYLATION AND ROLE OF PROTEIN PHOSPHATASES IN AD BRAIN

Hyperphosphorylation of tau is thought to be the result of an imbalance in the regulation of protein kinases and protein phosphatases (PP). Recently, it was reported that tyrosine 18 or tyrosine 394 on tau are phosphorylated by the src family tyrosine kinase fyn or non-receptor tyrosine kinase c-Abl.[15,16] However, known functions associated with phosphorylation sites on tau have been reported for serine and threonine residues only, suggesting a crucial role for serine and threonine kinases and PP in AD.

There are more than 10 serine/threonine protein kinases, such as extracellular signal-regulated kinase (ERK) 1/2, cell division cycle (cdc)-2 kinase, cyclin-dependent kinase (cdk)-2, cdk-5, calcium–calmodulin-dependent protein kinase II (CaMKII), protein kinase (PK) A, PKC, casein kinase I/II, and $P^{110MARK}$, that have been shown to phosphorylate tau *in vitro*. Of these, glycogen synthase kinase (GSK) 3β is the most likely to be involved in the abnormal hyperphosphorylation of tau in AD brain.[17]

It has been reported that the PPs that participate in dephosphorylation of tau in AD are PP1, PP2A, PP2B, and PP5, but not PP2C.[18] Of these, PP2A is the most effective phosphatase in dephosphorylating hyperphosphorylated tau isolated from AD brains.[19] It has been shown that PP2A accounts for approximately 71% of the total tau phophatase activity in the human brain, with the activity of PP2A being significantly decreased in AD brains.[20,21]

REGULATION OF PP2A ACTIVITY *IN VIVO*

The PP2A holoenzyme consists of two common subunits, namely catalytic subunit C (PP2Ac) and a structural scaffolding subunit A, together with various a regulatory B subunits.

The activity of PP2A is regulated by several post-translational modifications, including phosphorylation of tyrosines[22] or threonines,[23] which inactivate PP2A, and methylation of the carboxyl-termminal leucine Leu[309], which activates PP2A.[24]

In addition, PP2A activity is regulated by two inhibitor proteins, termed I_1^{PP2A} and I_2^{PP2A}. Both inhibit PP2A in a non-competitive manner and exhibit apparent K_i values in the nanomolar range.[25] Recently, I_1^{PP2A} and I_2^{PP2A} were cloned from the human brain and it was demonstrated that overexpression of I_1^{PP2A} and I_2^{PP2A} led to the hyperphosphorylation of tau via inhibition of PP2A activity *in vitro*.[26]

INCREASED I_1^{PP2A} AND I_2^{PP2A} mRNA LEVELS IN AD BRAIN

We investigated the involvement of the PP2A inhibitors I_1^{PP2A} and I_2^{PP2A}, particularly I_2^{PP2A}, in neurofibrillary pathology in AD.[27] The expression of I_1^{PP2A} and I_2^{PP2A} was determined by digoxigenin-labeled *in situ* hybridization histochemistry in AD and control brains. Comparison of the levels of I_1^{PP2A} and I_2^{PP2A} between AD and control subjects revealed a disease-associated increase of neuronal I_1^{PP2A} and I_2^{PP2A} mRNA in the temporal cortex of AD brains (Fig. 1a). The relative expression of both I_1^{PP2A} and I_2^{PP2A} mRNAs after normalizataion against GAPDH mRNA was approximately 25% higher ($P < 0.001$) in the temporal cortex of AD brains compared with control (Fig. 1b; data not shown for I_1^{PP2A}).

REDISTRIBUTION OF I_2^{PP2A} FROM THE NUCLEUS TO THE CYTOPLASM OF NEURONS FROM AD BRAIN

The subcellular localization of I_2^{PP2A} has been reported in various cultured cells. For example, I_2^{PP2A}, which is the same as SET/template-activating factor (TAF)-1β, is mainly localized in the nucleus.[28–31] However, TAF1β has been shown to be cleaved and the amino terminal cleaved half, which has PP2A inhibitory activity similar to the full-length of I_2^{PP2A}, is localized in the cytoplasm.[30]

The subcellular distribution of I_2^{PP2A} was investigated in AD and control brains by immunohistochemistry using a specific polyclonal antibody (R-42187) that recognizes the amino terminal region of I_2^{PP2A}.[27] Surprisingly, I_2^{PP2A} was translocated from the nucleus to the cytoplasm in many neuronal cells in the temporal cortex of AD brains (Fig. 2a). The number of neurons in the temporal cortex of exhibiting translocation of I_2^{PP2A} from the nucleus to the cytoplasm was counted and compared between AD and control brains. It was found that the ratio of the number of neurons with immunonegative nuclei to those with nuclei immunopositive for I_2^{PP2A} was more than sixfold greater in AD than control brains (Fig. 2b; $P < 0.05$). In the cerebellum, the subcellular localization of I_2^{PP2A} was similar between AD and control brains (Fig. 2c).

CLEAVAGE OF I_2^{PP2A} IN THE TEMPORAL CORTEX OF AD BRAIN

To biochemically confirm the results of immunohistochemical analysis, western blots were preformed using nuclear and cytosol fractions prepared from temporal cortices of seven AD and seven control brains.[27] Consistent with the immunohistochemical findings, the signal for I_2^{PP2A} in the nuclear fraction was reduced ($P < 0.05$) in AD compared with control

brains. In the cytosol, the 39 kDa I_2^{PP2A} was cleaved and fragment levels were higher in samples from AD brains compared with control. The signal for the 39 kDa band in the cytosolic fraction was decreased in AD brains ($P < 0.05$). A major cleavage product, the approximately 20 kDa I_2^{PP2A} polypeptide, which was seen in the cytosolic but not the nuclear fraction, appeared in few control samples but was found in most samples from AD brain (Fig. 3). Levels of the 20 kDa polypeptide were significantly higher in AD cytosol compared with control ($P < 0.05$).

The same study was performed using nuclear and cytosol fractions from the cerebellum.[27] In the cerebellum, there was no significant difference in the expression of I_2^{PP2A} between AD and control brain (data not shown), suggesting that this cleavage of I_2^{PP2A} was limited to areas of the brain that develop neurofibrillary pathology.

COLOCALIZATION OF PP2A INHIBITORS WITH PP2A CATALYTIC SUBUNITS AND WITH HYPERPHOSPHORYLATED TAU IN NEURONAL CYTOPLASM

The increased levels of I_2^{PP2A} mRNA, cleavage of I_2^{PP2A} protein, and its translocation from the nuclear to the cytoplasmic compartment in neurons in AD brain prompted us to investigate whether PP2A inhibitors were involved in the hyperphophorylation of tau in AD. We performed double-labeled immunohistochemical studies using specific antibodies against I_2^{PP2A} (R-42187), PP2A catalytic subunit, and tau abnormally hyperphosphorylated at $Ser^{262/356}$ (12E8).[27] We found that I_2^{PP2A} was colocalized with the PP2A catalytic subunit in the neuronal cytoplasm in AD brains (Fig. 4a). In addition, we found that I_2^{PP2A} was colocalized in early or middle-stage neurofibrillary tangles of abnormally hyperphosphorylated tau in the neuronal cytoplasm (Fig. 4b). The neurons that expressed I_2^{PP2A} mainly in the nucleus did not exhibit phosphorylated tau immunoreactivity, indicating that the I_2^{PP2A} in the cytoplasm was probably responsible for tau hyperphosphorylation.

WHY IS THE EXPRESSION OF I_2^{PP2A} INCREASED IN AD BRAIN AND HOW DOES I_2^{PP2A} TRANSLOCATE FROM THE NEURONAL NUCLEUS TO THE CYTOPLASM IN AD BRAIN?

Possible mechanisms and a new therapeutic target for AD

In an unpublished study (H. Tanimukai *et al*., unpubl. data, 2004, 2007), we have investigated why the expression of I_2^{PP2A} is increased in AD brain and how I_2^{PP2A} translocates from neuronal nucleus to the cytoplasm in AD brain. To address the first question, SH-SY5Y neuroblastoma cells were stimulated with 3 μmol/L Aβ(1–42) for 0, 2, 4, 24, and 48 h. After stimulation with Aβ(1–42), cell lysates were collected and the expression of I_2^{PP2A} was investigated by western blot. The preliminary evidence indicates that the expression of the I_2^{PP2A} holoprotein (molecular weight 39 kDa) was increased in a time-dependent manner (Fig. 5; H. Tanimukai *et al*., unpubl. data, 2007), suggesting that Aβ(1–42) stimulation is probably responsible for the increase in I_2^{PP2A} expression.

The activity of PP2A against tau protein is not likely to be compromised simply as a result of increased I_2^{PP2A} expression in the neuronal nucleus because an interaction between I_2^{PP2A} and the PP2A catalytic subunit is needed for a reduction in PP2A activity in the neuronal cytoplasm, where the PP2A catalytic subunit and tau mainly localize. So, we speculated that the translocation of I_2^{PP2A} from the neuronal nucleus to the cytoplasm was

the most crucial event in the hyperphosphorylation of tau following on from a reduction in PP2A activity.

To address the second question, recombinant I_2^{PP2A} [26] was digested with brain extract from AD cases and the digested product (approximately 20 kDa), which was similar to that seen in AD brain, was analyzed by mass spectrometry and amino terminal sequencing. As expected, the digested product was from the amino terminal half or greater and the digestion site was located between amino acids 175 and 176 on I_2^{PP2A} (H. Tanimukai *et al*., unpubl. data, 2004). Because the sequence between amino acids 168 and 181 (KRxxxxxxxxxRKR) on I_2^{PP2A} is considered a plausible nuclear localization signal (NLS),[32] we speculate that the translocation of I_2^{PP2A} from the nucleus to cytoplasm is due to a break in the NLS located on the I_2^{PP2A} sequence by an as yet unidentified protease(s) that we will temporarily call protease X. Previously, we reported an increase in the I_2^{PP2A} cleavage activity by protease X in AD brain compared with control brain.[27] In addition, Chohan *et al.* reported that overexpression of I_2^{PP2A} resulted in abnormal hyperphosphorylation of tau in cultured cells and that this was observed only when a subcellular shift of I_2^{PP2A} occurred from the nucleus to cytoplasm that was accompanied by cleavage of I_2^{PP2A} into the 20 kDa fragment.[33]

Taken together, these data indicate that a potential novel therapeutic target would be to inhibit the translocation of I_2^{PP2A} from the neuronal nucleus to the cytoplasm (Fig. 6). Although further investigation is required to identify protease X, the activity of which is elevated in AD brain, inhibitors of protease X may also turn out to be new therapeutic drugs for AD.

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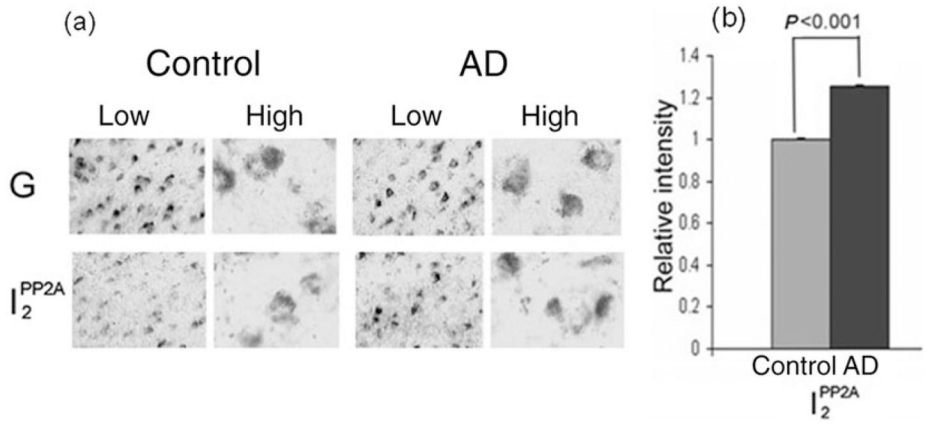

Figure 1.

Expression of I_2^{PP2A} mRNA in Alzheimer disease (AD) and control brain.[27] (a) The I_2^{PP2A} signal was significantly elevated in AD brain (temporal cortex) compared with control brain ($P < 0.001$), whereas the GAPDH signal (G) was similar between the two. Differences between AD and control brains were analyzed statistically by Student's *t*-test. High, high magnification (original magnification ×630); Low, low magnification (original magnification ×200). (b) The I_2^{PP2A} signals from five AD and five control cases were quantified and normalized against the GAPDH signal. Data show the mean ± SEM. Modified and reproduced with the permission from the American Society for Investigative Pathology from Tanimukai *et al.*[27]

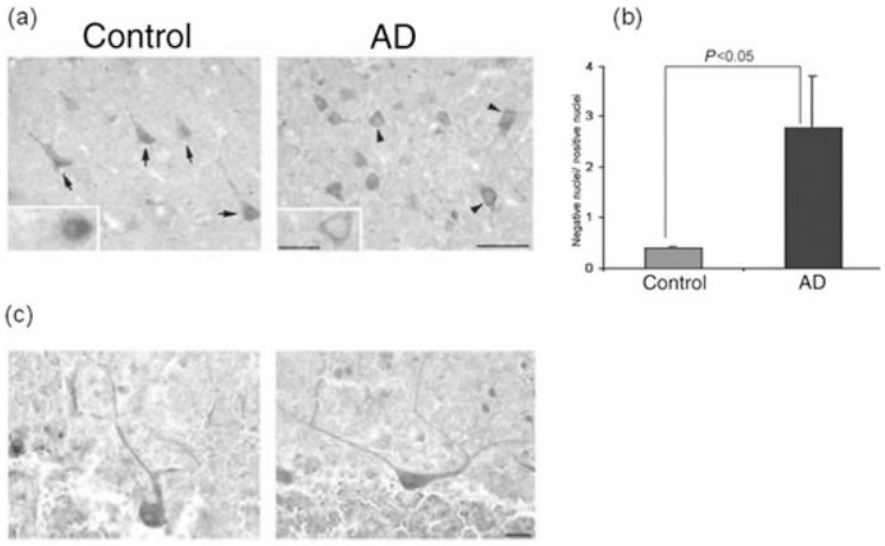

Figure 2.

Subcellular localization of I_2^{PP2A} in Alzheimer disease (AD) and control brains.[27] (a) I_2^{PP2A} was predominantly expressed in the nucleus (arrows) of neurons in the temporal cortex from control brain, but was translocated from the nucleus to cytosol (arrowheads) in AD brain. (b) Ratio (mean ± SEM) of neurons with immunonegative to immunopositive nuclei. In AD brains, the number of neurons in the temporal cortex showing the translocation of I_2^{PP2A} from the nucleus to a cytoplasmic localization increased markedly ($P < 0.05$). Differences between AD and control cases were analyzed statistically by Student's *t*-test. (c) In contrast, the subcellular localization of I_2^{PP2A} in the cerebellum was restricted to the nucleus in both AD and control brain. Modified and reproduced with the permission from the American Society for Investigative Pathology from Tanimukai *et al.*[27]

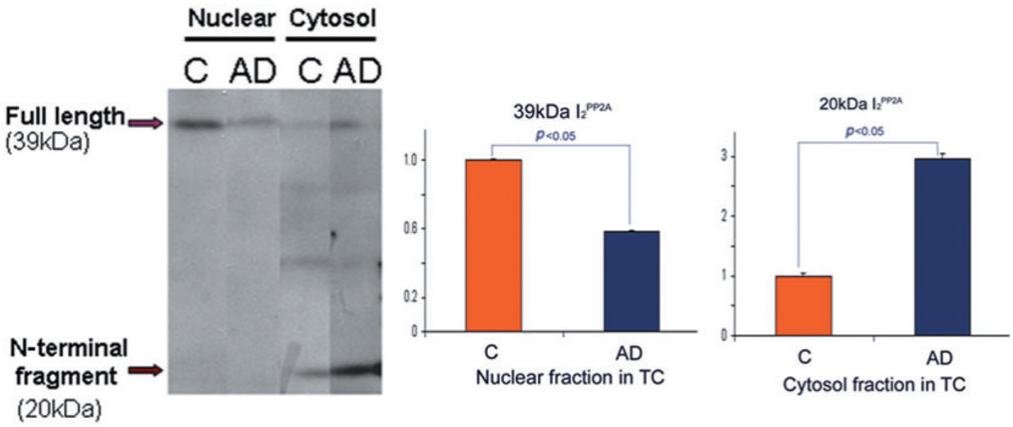

Figure 3.

Cleavage and distribution of I_2^{PP2A} in nuclear and cytosolic fractions of the temporal cortex (TC) in Alzheimer disease (AD) and control (C) brains.[27] Levels of I_2^{PP2A} in the nuclear fraction were decreased in AD compared with control brain. In contrast, the 39 kDa I_2^{PP2A} in the cytosolic fraction was decreased in AD brain, but the approximately 20 kDa fragment of I_2^{PP2A} was significantly increased in AD compared with control brain (*$P < 0.05$). Differences between AD and control brains were analyzed statistically by Student's *t*-test. Modified and reproduced with the permission from the American Society for Investigative Pathology from Tanimukai *et al.*[27]

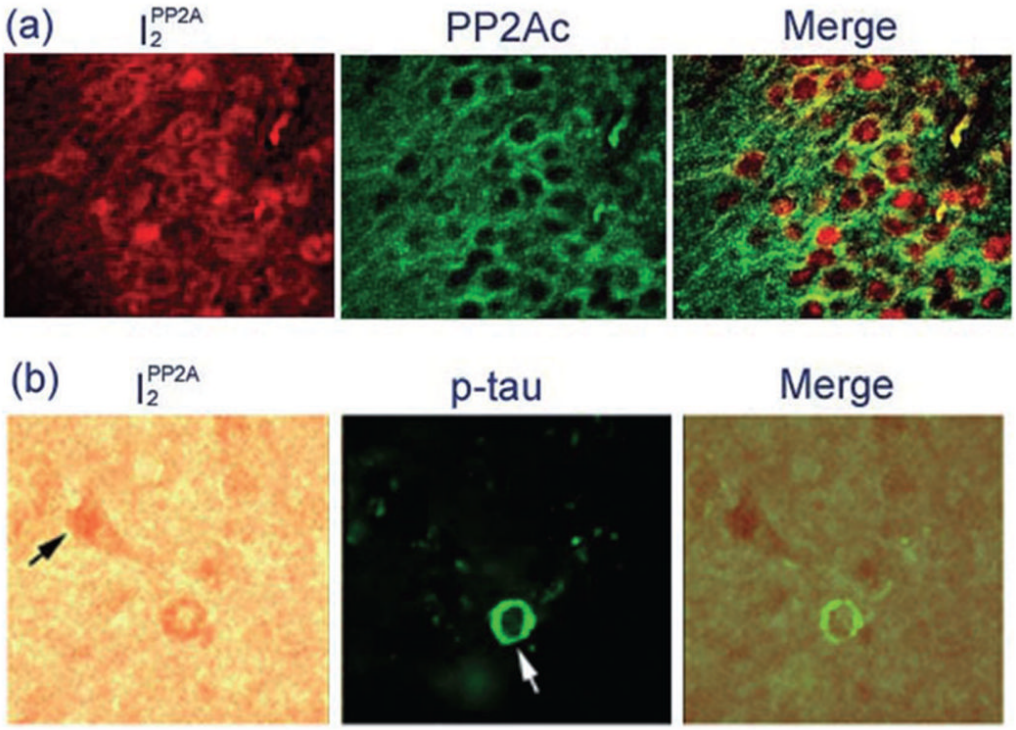

Figure 4.

Colocalization of I_2^{PP2A} with protein phosphatase 2A (PP2A) and with phosphorylated tau in Alzheimer disease (AD) brain.[27] (a) Subcellular localizations of I_2^{PP2A} and the PP2A catalytic subunit in AD hippocampus. I_2^{PP2A} was colocalized with PP2A in the neuronal cytoplasm. (b) I_2^{PP2A} was colocalized with phosphorylated tau in mostly early stage (white arrow) to middle stage (white arrowhead), where neurofibrillary changes were seen using a phosphorylation-dependent antibody to abnormally hyperphosphorylated (p-) tau in the AD temporal cortex. Neurons that expressed I_2^{PP2A} mainly in the nucleus did not colocalize with p-tau (black arrows). Modified and reproduced with the permission from the American Society for Investigative Pathology from Tanimukai *et al.*[27]

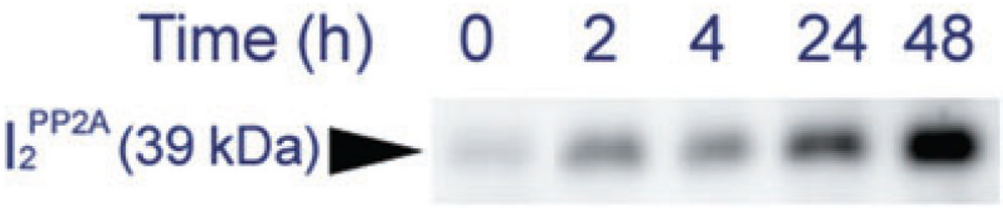

Figure 5.
Expression of I_2^{PP2A} SH-SY5Y neuroblastoma cells after stimulation with 3 μmol/L Aβ(1–42) for 0, 2, 4, 24, and 48 h. The induction of the expression of I_2^{PP2A} by 3 μmol/L Aβ(1–42) was time dependent.

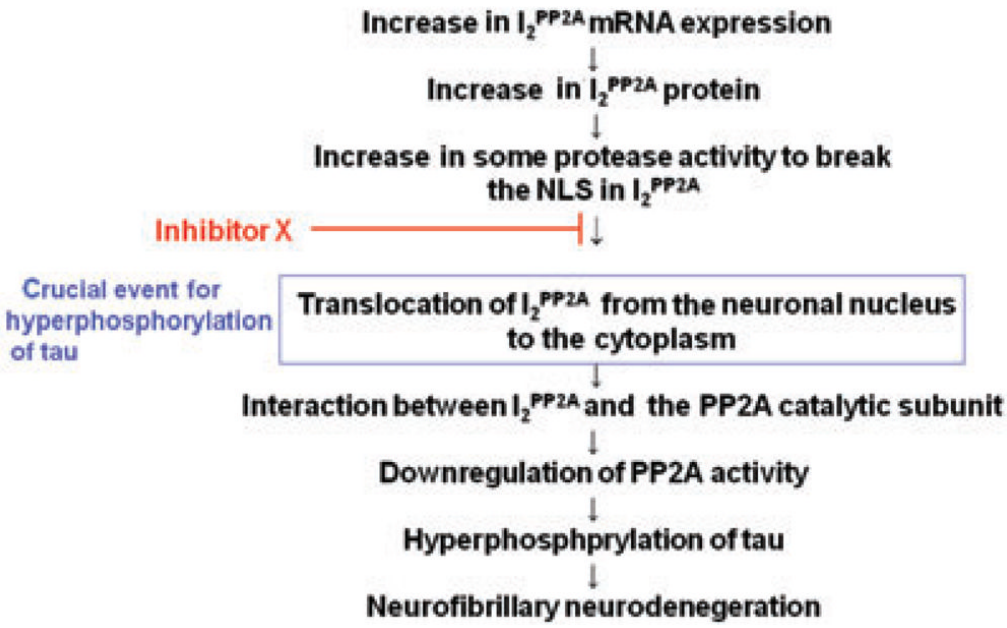

Figure 6.
Schematic of a proposed therapeutic strategy for the neurofibrillary neurodegeneration in Alzheimer's disease. NLS, nuclear localization signal; PP2A, protein phosphatase 2A.