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Altered Intracellular Distribution of PrP^C and Impairment of Proteasome Activity in Tau Overexpressing Cortical Neurons

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Abstract. The microtubule associated protein tau plays a crucial role in Alzheimer's disease and in many neurodegenerative disorders collectively known as *tauopathies.* Recently, tau pathology has been also documented in prion diseases although the possible molecular events linking these two proteins are still unknown. We have investigated the fate of normal cellular prion protein (PrP^C) in primary cortical neurons overexpressing tau protein. We found that overexpression of tau reduces PrP^C expression at the cell surface and causes its accumulation and aggregation in the cell body but does not affect its maturation and glycosylation. Trapped PrP^C forms detergent-insoluble aggregates, mainly composed of un-glycosylated and mono-glycosylated forms of prion protein. Interestingly, co-transfection of tau gene in cortical neurons with a proteasome activity reporter, consisting of a short peptide degron fused to the carboxyl-terminus of green fluorescent protein (GFP-CL1), results in down-regulation of the proteasome system, suggesting a possible mechanism that contributes to intracellular PrP^C accumulation. These findings open a new perspective for the possible crosstalk between tau and prion proteins in the pathogenesis of tau induced-neurodegeneration.

Keywords: Neurodegenerative diseases, prion, proteasome activity reporter, tau, tauopathies

INTRODUCTION

Tau are a protein family associated to microtubules and their correct functioning is important for regulating microtubule dynamics, axonal transport of organelles and vesicles, Golgi positioning, and neurite outgrowth [1]. The proper function of tau depends upon a precise equilibrium between the six isoforms, its state of phosphorylation, and its structural integrity. An impairment of these parameters may cause detrimental effects eventually leading to neurodegeneration. Indeed, neuronal loss and abundant tau-positive neurofibrillary lesions constitute a defining neuropathological characteristic of Alzheimer's disease (AD) and also to a number of other dementing disorders, such as Pick's disease, progressive supranuclear palsy, corticobasal degeneration and familial frontotemporal dementia, and Parkinsonism linked to chromosome 17 (FTDP-17) [2]. In these diseases, tau pathologies are accompanied by synaptic failure, transport defects, Golgi fragmentation, loss of proteasome activity, protein aggregation, and neuronal loss [3–5].

AD and Creutzfeldt-Jakob disease (CJD) share several neuropathological characteristics. In particular, tau deposits have been observed in CJD patients with the mutation of amino acid 208 of prion protein and certain Gerstmann-Straussler-Scheinker cases, near or around PrP plaques [6, 7]. Although mutations of tau

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gene do not increase the risk of prion diseases [8], the finding that human tau protein interacts *in vitro* with the N-terminal portion of PrP^C and some mutated form of prion protein [9, 10] raises the question of whether prion protein might be directly or indirectly involved in the pathogenesis of neuronal dysfunction caused by tau.

Although some evidence suggests that tau might be involved in the pathogenesis of transmissible spongiform encephalopathies (TSEs) playing a role as a possible "end-stretch of prion-induced neurodegeneration" [11], direct evidence of it is still lacking.

The pathogenic mechanism of prion diseases or TSEs is based on the molecular conversion of the normal cellular PrP^C into the pathogenic isoform, termed as scrapie (PrP^{Sc}). This isoform accumulates in the brain, causing extensive neurodegeneration with an inevitably fatal outcome [12, 13]. The cellular prion protein PrP^C is a cell-surface glycoprotein normally imported into the endoplasmic reticulum (ER) and targeted to the outer leaflet of the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor [14]. It is mainly expressed in the central nervous system [15] and, to a lesser extent, in extra-neural tissues [16, 17]. PrP^C is predominantly found on the plasma membrane of dendrites, including spines, in transport vesicles either dendritic or axonal, endosomes, and axolemma. Although it is clear that the expression of PrP^C is mandatory for the pathogenesis of prion diseases, its normal function still remains enigmatic. In the majority of prion diseases, there is a correlation between the accumulation of misfolded PrP, formation of infectious prions and neurodegeneration. However, as for other neurodegenerative diseases, such as AD, Parkinson's, and polyglutamine diseases, there is an ongoing debate concerning the nature of the neurotoxic species.

Wild type PrP^C is degraded by the lysosomes, although a small proportion of PrP was found to be degraded in the cytosol by the proteasome [18, 19]. This cytosolic fraction, whose function is to date unknown, has been shown to misfold, form neurotoxic oligomers, and aggregate in cell models and transgenic mice [19]. In the cytosol, prion protein may be involved in a direct or indirect interaction with tau.

Here we use a model of tauopathy consisting of primary cortical neurons overexpressing the longest human (4 R) tau isoforms [20] to study tau-prion interplay. We find a marked alteration of prion protein traffic induced by tau overexpression. Surface depletion of PrP^C is accompanied by prion protein accumulation inside the cells and impairment of the proteasome pathway.

MATERIAL AND METHODS

Antibodies and reagents

Mab 7A12 [21] was a generous gift from Dr. Sy (Case Western Reserve University, Cleveland, OH, USA). Rabbit polyclonal anti tau antibody was from DAKO (code A0024). Mouse anti Thy-1.1 monoclonal antibody (clone MRC-OX0.7) was from Abcam. Mouse anti-myc IgG 9E10 was purchased from Santa Cruz (Santa Cruz, CA, USA). Mouse anti-β actin was from Affinity Bioreagents (ABR; Golden, CO, USA). Secondary antibodies donkey anti-mouse IgG horseradish peroxidase (HRP) and donkey anti-rabbit IgG HRP were from Jackson Immunoresearch (Suffolk, UK). PNGase F was from New England Biolabs and PI-PLC was from Sigma-Aldrich.

Plasmids and vectors

Ad-Lac-Z (coding for β-galactosidase) and Ad-myc tau adenoviral vectors have been described [22]. Plasmid encoding full-length tau cDNA was generated by PCR using tau40pSG5 vector as template [23] with primers containing Eco-RI and Bam-HI for insertion into Eco-RI-Bam-HI cut pIRES-DsRed2 vector (Clontech, Takara Bio USA). Synapsin-GFP tau was generated by cloning the AgeI (blunt)-BamHI fragment from pAcGFP-C1 vector (Clontech, Takara, Bio USA) coding for GFP-tau (1–441) into EcoRI (blunt)-BamHI cut pHSYN-MH4-I vector, gently provided by Dr. Kugler (Neuro-Regeneration Laboratory, University of Tübingen, Germany). pIRES-tau plasmid was generated by cloning Eco-BamHI tau (1–441) fragment from GFP-tau (1–441) vector into Eco-BamHI cut pIRES-Ds-Red2 vector. All new generated plasmids were verified by digestion with restriction enzymes and sequencing. Plasmid GFP-CL1 was gently provided by Dr. Nonaka (Molecular Neurobiology, Tokyo Institute of Psychiatry).

Cell cultures, transduction, and transfection

Cortical neurons were prepared from embryonic day 17–18 (E17/E18) embryos from timed pregnant Wistar rats (Charles River), as previously reported [24]. In detail, the cortex was dissected out in Hanks' balanced salt solution buffered with HEPES and dissociated via trypsin/EDTA treatment. Cells were plated at 1×10^6 cells on 3.5 cm dishes precoated with poly-DL-lysine. After 2 days of culturing in neurobasal medium with B-27 supplement and glutamax, cytosine arabinofuranoside was added to reduce glial proliferation. Half of the medium was changed every 3–4 days.

Transfection of cortical neurons with GFP, GFP-tau, GFP-CL1, and pIRES2-Ds-Red vectors was performed on 7–10-day-old cortical neurons cultured on 24 mm coverslips using lipofectamine (Life Technologies) according to the manufacturer's direction. Transduction of cortical neurons with adenoviral particles for myc-tau and Lac-Z was performed on 7–10-day-old cultures as reported [22]. In brief, the volume of the medium was reduced to one-third and the recombinant adenovirus was added at an indicated MOI. After allowing the virus to adsorb for 1 h, the medium was rendered back to its original volume. Neurons were further cultured up to 8–12 DIV when the experiments were performed.

Immunofluorescence staining

Immunofluorescence was carried out as described [25]. For surface staining of PrP^C, cells were rinsed with ice-cold PBS and incubated for 1 h at 4 °C with MAb 7A12 diluted in Opti-MEM (Invitrogen) containing 0.2 mg/ml BSA. After washing with ice-cold PBS, cells were fixed in 4% paraformaldehyde for 10 min at room temperature. AffiniPure donkey anti-mouse TRITC IgG (Jackson Immunoresearch) was used as secondary antibodies. Nuclei were stained with Hoechst 33258 (Sigma) 0.5 μg/ml in PBS for 5 min. Samples were examined under a confocal laser scanning microscope (Leica SP5, Leica Microsystems, Wetzlar, Germany) equipped with 63×1.3–0.6 oil-immersion objective (optical section, 1 μM). Images of triple-labelled samples were recorded with simultaneous excitation and detection of both dyes to ensure proper image alignment. Optical sections were stereo-pair and three-dimensional reconstituted. To correct for possible crosstalk resulting from overlapping excitation and emission spectra of the dyes used, when necessary, recorded images were corrected using the MultiColor analysis package software by Leica.

Immunoprecipitation. Cortical neurons were lysed in extraction buffer containing 10 mmol/L Tris–HCl pH 7.6, 100 mmol/L NaCl, 10 mmol/L EDTA, 0.5% Nonidet P40, 0.5% sodium deoxycholate, proteases inhibitor cocktail set III (0.1 mmol/L AEBSF hydrochloride, 0.5 μmol/L aprotinin, 5 μmol/L bestatin, 1.5 μmol/L E-64, 10 μmol/L leupeptin, 1 mmol/L pepstatin A) and 1 μmol/L phenylmethylsulfonyl fluoride. Lysates were then centrifuged at 4°C for 15 min at 12.000 × g and the supernatants were pre-cleared using 5 ml of protein G (Invitrogen) for 1 h at 4°C. The clarified cell extracts were immunoprecipitated by cross-linking the Ig's antibodies to Protein G Sepharose beads (Boehringer Mannheim) according to the manufacturer's instructions, using anti prion protein mab 7A12 and non relevant monoclonal mouse IgG antibody as control.

Western blot analysis and enzymatic reactions

For intracellular prion detection, Ad-Lac-Z and Ad-myc-tau transducted cortical neurons were rinsed twice with ice-cold PBS and lysed for 30 min at 4°C in ice-cold extraction buffer. Nuclei and large debris were removed by centrifugation at 290 g for 10 min at 4°C. The supernatants were then precipitated with 5 volumes of MeOH at –20°C for 2 h. After centrifugation (16.000 g for 15 min), pellets were dissolved in 4×Sample Buffer (500 mmol/L Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 40 mmol/L dithiothreitol and 0.02% bromphenol blue) and heated at 95°C for 5 min.

Equal amount of proteins were separated by SDS-PAGE in 12% or NuPAGE pre-cast 10% acrylamide gels (Invitrogen) and transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, Little Chalfont, UK) for 16 h at 30 V. Densitometric measurements were performed on a VersaDoc Imaging System (BioRad, Milano, Italy).

For enzymatic deglycosylation, clarified cell lysates were methanol-precipitated, resuspended in denaturing buffer (0.5% SDS, 1% β-mercaptoethanol) and boiled for 10 min, before digestion with PNGase-F (1000 units in 1% Nonidet P-40, 25 mM sodium phosphate, pH 7.5). For membrane bound prion detection, phosphatidylinositol-specific phospholipase C (PI-PLC) (ICN) digestion of Ad-Lac-Z and Ad-myc-tau transducted cortical neurons was performed. Cortical neurons were cultured for 2 h in Opti-MEM containing 1 U/mL PI-PLC. Supernatants containing PI-PLC released proteins were MeOH precipitated and immunoblotted. All experiments were performed in triplicate.

RESULTS

Endogenous tau co-localizes with prion protein in primary neuronal cells

It has been recently reported that human tau *in vitro* interacts with the N-terminal portion of PrP^C [9]. In order to check whether this interaction also occurs *in vivo*, we have undertaken indirect immunofluorescence and co-immunoprecipitation assays in primary rat cortical neurons. As shown in Fig. 1A, immunofluorescence analysis using anti-prion protein mab 7A12 and anti-tau polyclonal antibodies reveals

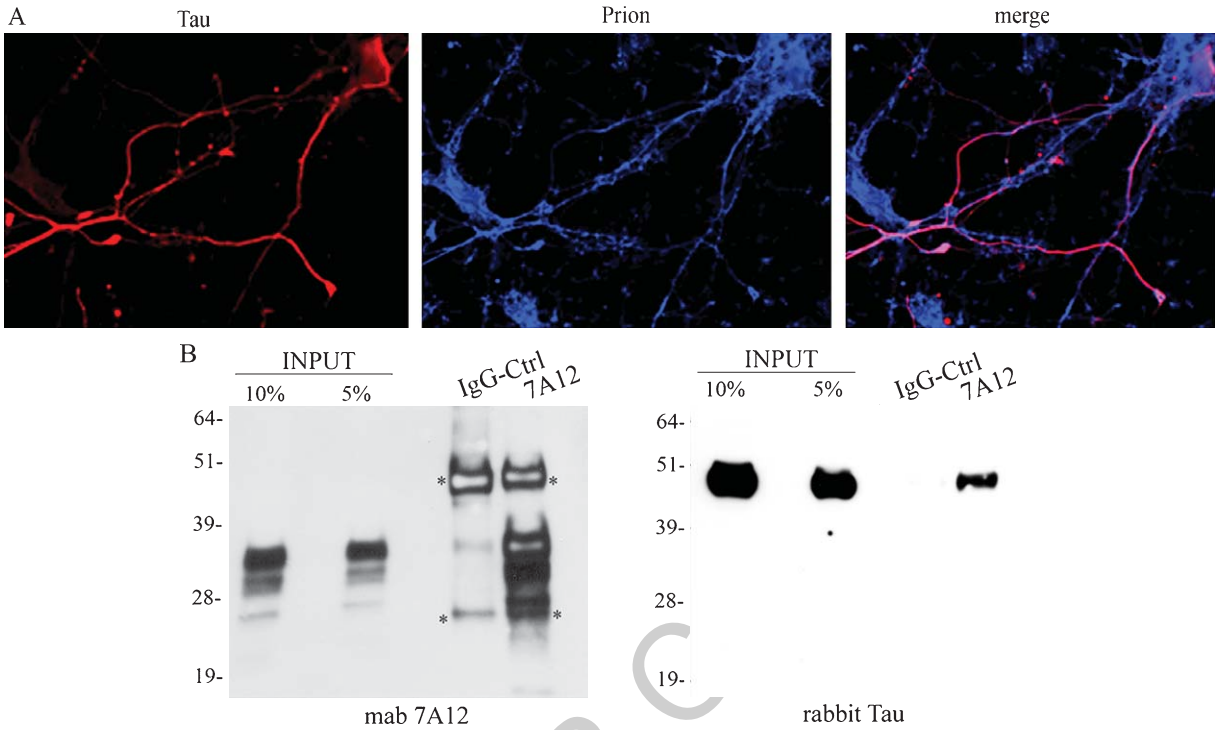

Fig. 1. *In vivo* interaction between prion and tau proteins in primary neuronal cells. A) Immunofluorescence staining of primary cortical neurons showing tau (red) visualized with rabbit anti-tau polyclonal antibodies and prion protein (blue) visualized with mab 7A12. Note that overlapping labeled areas are more evident in neurites than in cell bodies consistent with the prevalent axonal and *in vitro* dendritic expression of tau protein. B) Lysates from cortical neurons were first immunoprecipitated with anti prion mAb 7A12 or nonspecific mouse IgG as control, resolved on 12% SDS PAGE and then probed with anti-prion mAb 7A12 (left panel) and anti-tau polyclonal (right panel) antibodies respectively. Total protein extract (20 and 5% INPUT) was also loaded as positive internal control of electrophoretic mobility and immunoprecipitation efficiency. Asterisks denote the heavy and light immunoglobulin chains.

a strong signal of merged staining of both proteins mainly along the neurites, at the tip of them and, to a lesser extent, in the cell body. Tau staining is consistent with the reported distribution of this protein in cell cultures [26]. Co-immunoprecipitation of prion protein from cortical neuron lysates using mAb 7A12 followed by Western blot of the precipitants with anti-tau polyclonal antibody shows the presence of tau protein (Fig. 1B, right panel). This result suggests that tau and prion proteins may form a complex *in vivo*. It is of note that mAb 7A12 is specific to PrP^C residues 143–155 that are not involved in binding to tau [9]. No interaction is evident when a non-specific monoclonal mouse antiserum (IgG_{Ctrl}) is used for immunoprecipitation as control (Fig. 1B).

Effect of tau overexpression on prion protein cellular metabolism and distribution

In attempt to identify the biological significance of prion/tau interaction, we have overexpressed full-length tau (1–441) in primary neurons, bearing a myc-tag at the N-terminus (Ad-myc-tau), by adenoviral-mediated gene transduction [22]. Cortical neurons were transduced with a MOI of 30 of Ad-myc-tau 1–441 that, as previously reported, slightly, adversely affect survival [20]. Control neurons were transducted with Ad-Lac-Z. Western blot analysis, performed at different times post-transduction, reveals that in neurons overexpressing tau there are no changes either in the overall expression of prion protein either on its pattern of glycosylation (Fig. 2A). This latter is also confirmed *in vitro* by de-glycosylation with PNGase F digestion. Indeed, removal of N-linked glycans yields in the appearance of a major band of 29 kDa, which is the full-length prion protein and a minor band migrating at 21 kDa both in Ad-LacZ and in Ad-myc-tau transducted neurons (Fig. 2A).

To investigate whether overexpression of tau might affect endogenous prion distribution in neurons, we have generated a vector coding for tau protein fused to GFP under the control of the neuronal specific

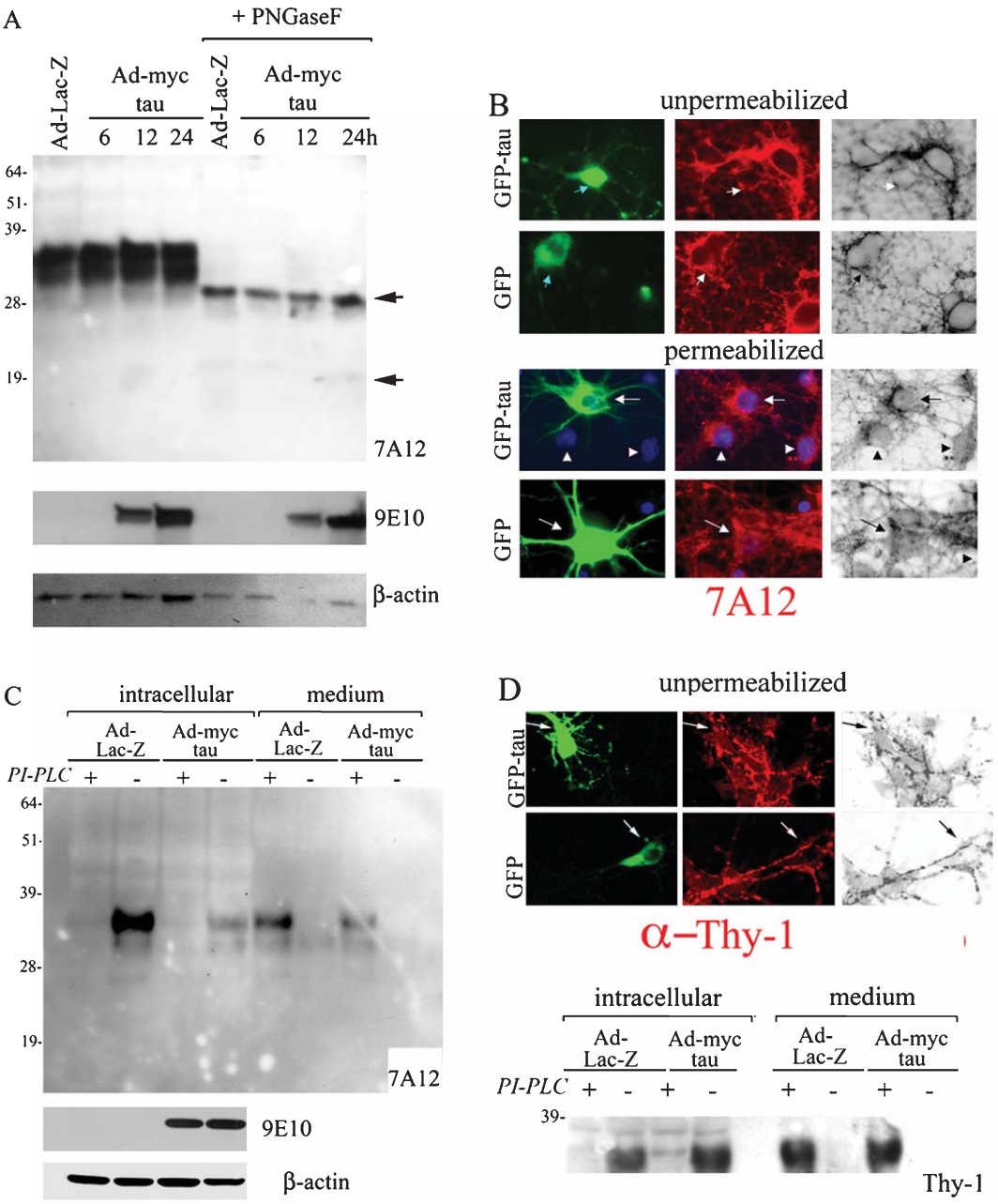

Fig. 2. Effect of tau overexpression on prion protein cellular metabolism and distribution. A) Total lysates derived from primary cortical neurons transducted with Ad-Lac-Z and Ad-myc-tau for the time indicated were treated or not for 3 h with PNGase F (1000 units). Blots were developed with anti-prion mab 7A12, anti-myc mab 9E10 to visualize exogenously expressed tau protein and mab anti β-actin used as normalization factor. The blots shown are representative of three different experiments. Arrows indicate the deglycosylated prion bands. B) Immunofluorescence analysis performed in fixed and permeabilized cortical neurons transfected with GFP-tau and GFP backbone vectors (green, arrow) using mab 7A12 to reveal PrP^C (red); arrowheads indicate non transfected neurons in permeabilized cells. The right panels report the prion protein red staining converted first to black and white and then inverted to white and black, using the channel mixer of Adobe Photoshop. C) Western blot of soluble intracellular and collected medium from Ad-Lac-Z and Ad-myc tau transducted cortical neurons treated with PI-PLC. PrP^C was detected with mab 7A12. D) Immunofluorescence analysis, performed as in panel B, of Thy-1 (red). Western blot of cellular lysates (intracellular) and collected medium from Ad-Lac-Z and Ad-myc tau transducted cortical neurons treated with PI-PLC visualized with mab anti-Thy-1. Molecular mass markers are given in kilodaltons. The blot shown is representative of three different experiments.

promoter synapsin. This plasmid allows to easily distinguishing neurons overexpressing tau in immunofluorescence analysis.

Control cortical cultures were transfected with the GFP backbone vector. It is worth noting that GFP does not bind to microtubules and does not affect transport and localization of vesicles and organelles [27]. As shown in Fig. 2B, a strong reduction of PrP^C surface fluorescence was detected in GFP-tau transfected neurons. By contrast, GFP-transducted control neurons do not manifest any change in membrane prion distribution. When cortical cells were fixed, permeabilized, and then examined for intracellular distribution, PrP^C signal is markedly increased and mostly concentrated in discrete perinuclear structures in the soma, suggesting tau-induced trapping of prion protein inside the cells (Fig. 2B). To obtain biochemical evidence for the altered distribution of PrP^C in tau overexpressing neurons, we treated cells with PI-PLC that is known to release GPI-anchored proteins [14]. The surface PrP^C (medium) and the intracellular PrP^C from Ad-Lac-Z and Ad-myc tau transducted cortical neurons were analyzed separately. As shown in Fig. 2C, the amount of prion protein released by PI-PLC digestion is ∼20–25% lower in neurons overexpressing tau. Quantification by densitometry reveals that the amount of prion protein in the medium after PI-PLC digestion in Ad-myc-tau transducted cortical neurons was $75 \pm 2.357\%$ ($n = 3$) compared to the amount of PrP^C in Ad-Lac-Z control neurons, which have been given a value of 100%. Surprisingly, when we analyzed the soluble intracellular remaining pool, we found a decrease in the amount of prion protein in neurons overexpressing tau compared to control cells. It is worth noting that the distribution at the cell surface and the intracellular amount of another GPI anchored protein, Thy-1, does not change in tau overexpressing neurons (Fig. 2D).

Overexpression of tau induces the formation of detergent insoluble PrP^C

To investigate whether a fraction of prion protein might form insoluble aggregates in tau overexpressing neurons, we studied the intracellular PrP^C solubility after lysing cells in non-ionic detergents containing Na-deoxycholate and Nonidet P-40. Cell lysates from Ad-Lac-Z and Ad-myc tau transducted cortical neurons were ultra-centrifuged to obtain a detergent-soluble supernatant and a detergent-insoluble pellet. These fractions were immunoblotted using mab 7A12 (Fig. 3A). In control cortical neurons, PrP^C is all detected in the detergent-soluble fraction. In sharp contrast, in Ad-myc tau neurons a consistent fraction (≈40%) of PrP^C accumulates in the insoluble fraction. In this fraction is also present tau protein, suggesting a possible co-aggregation (Fig. 3A). Densitometry of the three major glycosylated isoforms of prion protein shows that in the insoluble fraction there is an increase of the un-glycosylated and mono-glycosylated forms of ∼3 and 2.4 folds respectively (Fig. 3B).

Given that the intracellular structures in which prion are accumulating (see Fig. 2B) resemble Golgi apparatus, we performed a colocalization analysis with Golga-3, a member of the golgin family localized in Golgi and involved in its positioning [28]. Both in control and GFP-tau transfected neurons, intracellular prion protein partly co-localizes with Golga 3 (Fig. 3C). However, although in our experimental conditions Golgi apparatus does not seem to be greatly affected by tau overexpression [29], the intracellular amount of prion protein is greater in tau overexpressing neurons.

Proteasome activity is impaired in cortical neurons overexpressing tau

Accumulation of un-glycosylated and mono-glycosylated isoforms of prion protein in detergent-insoluble aggregates suggests a possible impairment of the proteasome machinery [19, 30, 31]. To investigate whether overexpression of tau might affect proteasome activity, we co-transfected both tau and GFP-CL1 reporter vector in cortical neurons. This is a proteasome activity reporter consisting of a short peptide degron fused to the carboxyl-terminus of the green fluorescent protein, which is known to be degraded by proteasome. GFP-CL1 vector is considered a valuable tool for the evaluation of the ubiquitin-proteasome dependent proteolysis in mammalian cells [32, 33]. To easily detect tau protein in co-transfected GFP-CL1 cultured neurons, tau cDNA was cloned into pIRES2-DSRed expression vector that allows the simultaneous expression of a protein of interest and the DsRed-Express2 protein from the same mRNA transcript. As shown in Fig. 4A, the level of GFP fluorescence is virtually absent in control cultured neurons co-transfected with GFP-CL1 and pIRES2-DSRed backbone vector, confirming that the sequence CL1 is well targeting GFP for degradation by proteasomes. The fluorescence intensity related to GFP protein expression dramatically increases, on the contrary, when cells were treated with the proteasome inhibitor MG132 (2 μM overnight) (Fig. 4A). Interestingly, when we examined the effect of tau overexpression on proteasome

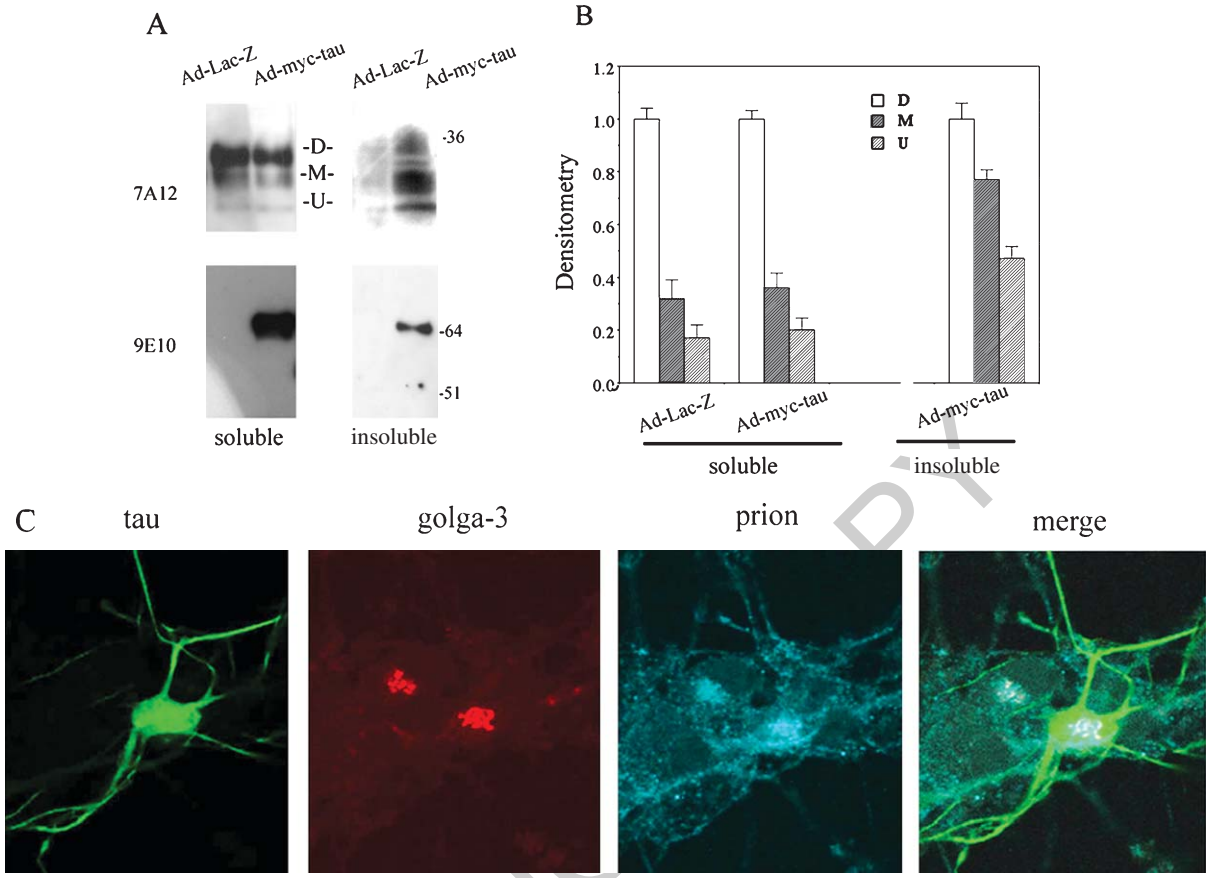

Fig. 3. Overexpression of tau induces aggregation of PrP^C. A) Postnuclear supernatans from Ad-LacZ and Ad-myc-tau transducted cortical neurons were ultracentrifuged to separate detergent-soluble and insoluble proteins. Western blot was performed with mab 7A12 and mab 9E10 to visualize prion and exogenous tau protein respectively. B) Densitometry of di-, mono-, and un-glycosylated PrP^C isoforms from three separate experiments are reported as fold of increase ± S.E. with respect to the values of the di-glycosylated isoform in the soluble fraction set as one-fold. C) Confocal analysis of cortical neurons transfected with GFP-tau (green) stained with anti Golga-3 (red) and anti-prion mab 7A12 (blue). The right panel denotes the overlay of signals (merge).

activity, we found a marked increase of GFP signal intensity in cortical neurons co-expressing GFP-CL1 and IRES2-DSRed-tau vectors (Fig. 4B), compared with the background fluorescence signal seen in cells transfected with IRES2-DS-Red backbone vector (Fig. 4A), indicating that cellular proteasome activity is inhibited.

DISCUSSION

We have presented evidence in this study that overexpression of the longest human tau isoform in cultured primary cortical neurons has a strong impact on prion trafficking, inducing a marked down-regulation of the cell-surface bound PrP^C and its accumulation in the cell body forming insoluble aggregates. Moreover, in these neurons the proteasome system is highly inhibited.

The pathological mechanisms of tauopathies are different and range from destabilization of cytoskeleton [34], to axonal transport inhibition [27], to abnormal bundling and accumulation of F-actin [35], to malpositioning and fragmentation of Golgi apparatus [36], and to synaptic dysfunction [37]. All these mechanisms alone or in combination, might possibly have an impact on prion trafficking. Among tau pathologies, we focused on 3R-tau/4R-tau unbalance since it is sufficient to cause neurodegeneration and dementia [38]. We propose three possible mechanisms, not mutually exclusive, to explain impairment of prion traffic.

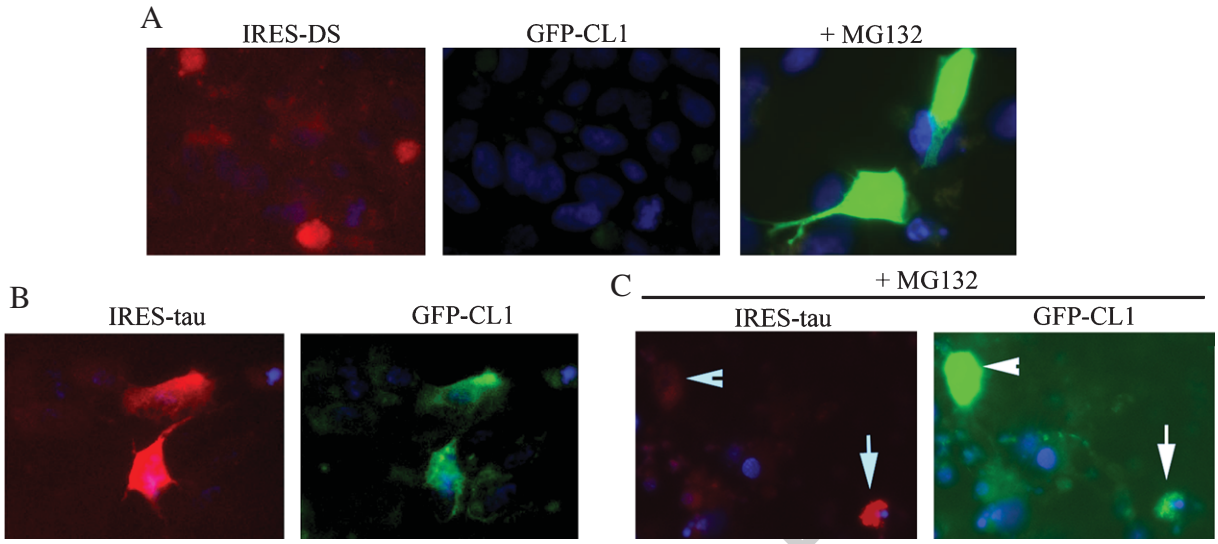

Fig. 4. Overexpression of tau impairs proteasome activity. Co-transfection of cortical neurons with (A) GFP-CL1 and pIRES-Ds-Red-2, (B) GFP-CL1 and pIRES-Ds-Red-2-tau vectors, and (C) GFP-CL1 and pIRES-Ds-Red-2-tau vectors treated with MG132. Cells were stained with Hoechst-33258 (blue). Note the intense fluorescence of cells transfected with GFP-CL1 and pIRES-Ds-Red-2 backbone vectors after inhibition of proteasomes with 2 μM MG132. Arrowhead in panel A, B, C indicates a neuron expressing only GFP-CLI and arrow indicates a suffering neuron expressing tau (red) and GFP (green) after treatment with MG132.

First, overexpression of tau may inhibit transport of prion protein vesicles, clogging its accumulation in the cell body [39–41]. As recently reported, PrP^C vesicles move bidirectionally along mammalian axons transported by kinesin and dynein motor proteins [42]. Tau binds to the microtubule surface and regulates protein traffic, influencing both kinesin and dynein motility both in the axon and in dendrites [43, 44]. The result of an impairment of the axonal transport would be the buildup of prion in the soma and its depletion at synapsis. Therefore, tau overexpression in neurons would result in defective PrP^C transport machinery in a manner resembling tau-induced altered AβPP trafficking [27, 45].

Second, overexpression of tau may hinder PrP^C entering into the ER, causing its accumulation in the cytosol. PrP^C contains a typical ER signal sequence of apparently average efficiency, which allows a fraction of prion to reside in the cytosol [46, 47]. This fraction could eventually directly interact with tau and accumulate in the cytosol. Interestingly, human tau protein forms a complex *in vitro* with the N-terminal portion of PrP^C and some mutated form of prion [9, 10] suggesting that a direct interaction may also occur *in vivo*. Indeed, tau and prion proteins are present in the same insoluble fraction and prion protein is mainly composed of the posttranslational not modified, unglycosylated isoform (Fig. 3A and B). However, additional experiments with antibodies that selectively react with the N-terminal signal peptide of prion will verify whether the prion signal peptide has been cleaved or not in tau overexpressing neurons.

Third, overexpression of tau can directly or indirectly induce misfolding of prion in the ER implying its retrotranslocation from the ER lumen to the cytosol. Misfolding may be part of an "amyloid cascade" induced by the overexpression of tau that mostly affects proteins prone to aggregate. This can explain the pathological accumulation of misfolded proteins, in AD or CJD [6, 48]. On the other end, misfolding can be the consequence of tau induced neuronal Golgi-fragmentation [29]. This event occurs before the formation of neurofibrillary tangles and may also influence prion traffic leading to its weak leak to the cytosol. A direct prion/ tau interaction in the cytosol, as previously mentioned, can induce misfolding and aggregation of the two molecules. The co-immunoprecipitation and co-localization studies showed in Fig. 1B seems to corroborate this last hypothesis although the effects on the aggregation, misfolding, and proteasome impairments are not necessary linked to a direct binding between the two proteins.

Much evidence suggests that, at the cell surface, prion interacts with proteins involved in cell adhesion and signaling [49, 50], as well as with the pathological isoform PrP^{Sc} [39]. Thus, inhibition of trafficking of

PrP^C to the plasma membrane by trapping prion in the ER by intrabodies or by drugs interfering with prion traffic have been proved to antagonize PrP^{Sc} formation and accumulation [25, 51]. Notwithstanding the fact that cell membrane prion is necessary for prion infectivity, it is worth noting that prion neuroinvasion is not hampered in transgenic mice overexpressing four-repeat tau [11]. The physiological role of PrP^C is controversial, but some studies suggest that, by modulating multiple signaling pathways, PrP^C is involved in neuritic outgrowth, neuronal survival, and integrity of synaptic function [52–54]. All these functions are impaired in neurons overexpressing tau. Therefore, it seems reasonable to suppose that some of them may be, in part, due to the observed altered intracellular distribution of prion. We have reported that overexpression of tau in primary neuronal cultures induces N-Methyl-D-Aspartate Receptor (NMDAR) mediated cell death and they are more vulnerable to N-Methyl-D-Aspartate (NMDA)-mediated toxicity [20 and unpublished observations]. Perturbations in the balance between synaptic and extra-synaptic NMDAR activity may account for tau induced NMDA vulnerability. However, since native PrP^C is neuroprotective, by virtue of its ability to inhibit NR2D subunits, tau-induced reduced expression of prion at plasma membrane would result in enhanced NMDAR activity, increased neuronal excitability and enhanced glutamate excitotoxicity as that observed in PrP null mice [55].

PrP^C has been reported to exert an anti-oxidative and cell protective role by modulating the activity of Cu^{2+}/Zn^{2+} superoxide dismutase, a key intracellular antioxidant enzyme [56]. Neurons overexpressing tau are more vulnerable to oxidative stress, likely as consequence of tau mediated inhibition of kinesin-dependent transport of peroxisomes [57]. Dislocation of prion protein may interfere with its anti-oxidative activity contributing to make neurons overexpressing tau more vulnerable to oxidative stress.

In conclusion, clarifying the role of PrP^C in tau models of neurodegeneration is of special interest not only for identifying new players of tau-mediated toxicity but also to understand the interplay between different neurodegenerative diseases.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=944).

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