Expression of Tau Produces Aberrant Plasma Membrane Blebbing in Glial Cells Through RhoA-ROCK-Dependent F-Actin Remodeling

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Abstract. Abnormal aggregation of Tau in glial cells has been reported in Alzheimer's disease (AD) and other tauopathies; however, the pathological significance of these aggregates remains unsolved to date. In this study, we evaluated whether full-length Tau (Tau441) and its aspartic acid⁴²¹-truncated Tau variant (Tau421) produce alterations in the normal organization of the cytoskeleton and plasma membrane (PM) when transiently expressed in cultured C6-glial cells. Forty-eight hours post-transfection, abnormal microtubule bundling was observed in the majority of the cells, which expressed either Tau441 or Tau421. Moreover, both variants of Tau produced extensive PM blebbing associated with cortical redistribution of filamentous actin (F-Actin). These effects were reverted when Tau-expressing cells were incubated with drugs that depolymerize F-Actin. In addition, when glial cells showing Tau-induced PM blebbing were incubated with inhibitors of the Rho-associated protein kinase (ROCK) signaling pathway, both formation of abnormal PM blebs and F-Actin remodeling were avoided. All of these effects were initiated upstream by abnormal Tau-induced microtubule bundling, which may release the microtubule-bound guanine nucleotide exchange factor-H1 (GEF-H1) into the cytoplasm in order to activate its major effector RhoA-GTPase. These results may represent a new mechanism of Tau toxicity in which Tau-induced microtubule bundling produces activation of the Rho-GTPase-ROCK pathway that in turn mediates the remodeling of cortical Actin and PM blebbing. In AD and other tauopathies, these Tau-induced abnormalities may occur and contribute to the impairment of glial activity.

Keywords: F-Actin remodeling, glial degeneration, membrane blebbing, Rho associated protein kinase, RhoGTPases, Tau, tauopathies

INTRODUCTION

It has been long documented that accumulation of fibrillary and nonfibrillary aggregates of Tau is a common neuropathological feature of Alzheimer's disease (AD) [1, 2] and of several types of subcortical

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dementias referred to as tauopathies [3]. In these disorders, not only are neurons affected by Tau aggregation, but this also occurs in several populations of glial cells, including astrocytes [4, 5], oligodendrocytes [3, 6], and microglia [7, 8].

From a physiological viewpoint, the formation of intracellular inclusions of Tau in glial cells might rely on the same mechanisms as in neuronal cells, thus entailing the same pathologic consequences. If so, degeneration of glial cells may also affect neuronal functioning and survival.

To further understand the mechanisms that underlie neurodegeneration caused by accumulation of Tau, in addition to several phosphorylated, mutated, or truncated Tau variants, overexpression of these entities in neuronal and non-neuronal cells has revealed several alterations in cell functioning prior to death [9–14].

Truncation of Tau at Asp⁴²¹ has been considered a relevant modification that increases the capacity of Tau to polymerize *in vitro*, but additionally composing the majority of neurofibrillary pathology in AD brains [15–17]. When this truncated variant of Tau was expressed in neurons, alterations in the functioning of several organelles, including mitochondria and the endoplasmic reticulum, was observed [10, 11]. Previously, we transiently expressed full-length Tau (Tau441) and Aspartic acid⁴²¹-truncated Tau (Tau421) in SH-SY5Y cells and in addition to alterations in the nuclear morphology, we observed increased microtubule bundling [18]. Overexpression of Tau and its capacity to promote microtubule bundling has been reported previously, but this capacity has been widely associated with physiological processes such as neuritic outgrowing and stability [19, 20]. In contrast, other reports on Chinese hamster ovary (CHO) cells [21, 22] and N2a neuroblastoma cells [23] concluded that overexpression of Tau inhibits kinesin-dependent trafficking of vesicles and disturbs the organization of intermediate filaments. Despite that some evidences of Tau-Actin interactions *in vitro* [24–27] and in cell systems [28–31] have been documented, little is known about any relationship under pathological conditions, with the exception of evidence that abnormally phosphorylated Tau produces neurodegeneration with the accumulation of filamentous Actin (F-Actin) and the formation of Actin-rich rods in *Drosophila* and mouse models [32]. From these findings, it was proposed that direct interaction between Tau and Actin may occur and mediate Tau-induced neurotoxicity in AD and related

disorders. On the other hand, in platelet-derived growth factor (PDGF)-stimulated 3T3-fibroblasts, the expression of Tau altered the remodeling of Actin-cytoskeleton through a mechanism in which Tau interacts with Src, which upregulates Src-tyrosine kinase activity and Src-dependent Actin remodeling [33].

Regarding the pathological significance of Tau accumulation inside glial cells, few reports have sought evidence of abnormalities by using the Tau-expressing cell approach. To contribute to this, primary cultured astrocytes from rat brains were adenoviral-vector-transfected to induce overexpression of the full-length Tau [34]. In this study, Tau produced an increase of microtubule bundling, but a selective reduction of stable detyrosinated microtubules. This effect was followed by alteration in the kinesin-dependent trafficking of organelles, Golgi apparatus fragmentation, and finally, cell death [34]. In contrast, transient overexpression of full-length Tau in cultured rat microglial cells produced an increase of Iba1, migration, and phagocytosis, all indicators of an activated state [35]. This physiological activation was also associated with the secretion of several interleukins, tumor necrosis factor- α (TNF- α), and nitric oxide (NO).

Due to the limited and contradictory information with respect to the pathological role of Tau in glial cells, we searched for more convincing evidence to clarify this unsolved issue. The data that we reported here indicate that transient expression of either Tau441 or Tau421 in C6-glial cells produces extensive microtubule bundling and abnormal blebbing of the plasma membrane (PM). These blebs may alter diverse physiological properties of the PM. Cell migration in a scratch-wound assay was altered in Tau-expressing C6-glial cells. To acquire insights into the plausible mechanism underlying these abnormalities, we found that the formation of PM blebs depends on remodeling of the F-Actin cytoskeleton, which is regulated upstream by the activation of RhoA (a small GTPase) and Rho-associated protein kinase (ROCK) signaling. All of these effects were initiated upstream by Tau-induced microtubule bundling, which may release microtubule-bound Guanine nucleotide exchange factor-H1 (GEF-H1) into the cytoplasm in order to activate its major effector, RhoA-GTPase. Taken together, these results may represent a new mechanism of Tau toxicity that contributes to the impairment of glial activity and neuronal dysfunction reported in AD and other tauopathies.

MATERIALS AND METHODS

Plasmid constructs

Plasmid preparation was described in our previous report [18]. Prior to transfection experiments, the integrity of pcDNA3.1 Zeo (-) plasmids containing the sequence to express either Tau441 or Tau421, as well as the empty vector, was confirmed by double sequence analysis. Green fluorescent protein (GFP)-fused Tau was produced from the pVLGT42 plasmid (GFP-Tau441).

Cell culture and transfection

The C6-glia cell line obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA) was grown in Dulbecco's Modified Eagle Medium (DMEM-F12); glucose (1/1) medium supplemented with 5% fetal bovine serum (FBS) (GIBCO-Life Technologies, Grand Island, NY, USA), 2 mM L-glutamine, and 100 U/mL penicillin, 100 µg/mL streptomycin, and was maintained under a humidified atmosphere of 5% CO₂ at 37°C. When this reached 50–60% confluence, C6-glia cells were changed to FBS-free Opti-mem specialized medium (GIBCO-Invitrogen) for 15 min and was then transiently transfected with the Tau constructs (plasmid pcDNA 3.1 Zeo (-), which contain the sequences for either Tau441 or Tau421, and plasmid pVLGT42, which contains the sequence for GFP-Tau441 by using the Lipofectamine®-PLUS™ reagent following the manufacturer's instructions (Invitrogen-Life Technologies, Carlsbad, CA, USA). Three h after transfection, the cells were post-incubated in fresh DMEM-F12 medium supplemented with 5% FBS and maintained for 48 h at 37°C. Thereafter, transfected cells were washed in ice-cold Phosphate buffered saline (PBS) pH 7.4 and processed for the different assays as described later. Under this protocol we obtained a transfection efficiency of 40%.

Cell viability

The 3- (4, 5-diMethylThiazol-2-yl)-2, 5-diphenyl Tetrazolium bromide (MTT) assay (Sigma-Aldrich, St. Louis, MO, USA) was employed to evaluate cell viability. This technique determines the ability of healthy cells to produce formazan from the cleavage of the MTT tetrazolium ring [36]. Briefly, enzyme-linked immunosorbent assay (ELISA) plates

with both transfected and non-transfected cells were washed with fresh culture medium and then incubated in fresh medium containing MTT (0.5 mg/mL) for 3 h at 37°C. The MTT medium was discarded and the cells were incubated in Dimethyl sulfoxide (DMSO) to dissolve the formazan aggregates. The intensity of the MTT products was read at 570 nm utilizing an ELISA microplate reader (iMark; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Immunofluorescence and confocal microscopy

For double, triple, and multiple immunofluorescence, transfected and non-transfected C6-glia cells were fixed with 4% paraformaldehyde in PBS (pH 7.4) at room temperature (RT) for 15 min and then permeabilized in 0.1% Triton X-100-PBS (PBSt). After blocking for 15 min at RT (in 0.5% gelatin and 1.5% FBS in PBS), cells were incubated with a variety of antibodies in pair-wise combinations, always mixing one mouse monoclonal-IgG with one rabbit polyclonal-IgG to avoid crosstalk and non-specific cross-reactions. Primary antibody incubations were conducted for 1 h at RT. Antibody specifications are described in Table 1. Pair-wise combinations, as well as multiple labeling with fluorescent markers, are depicted in the figures. Secondary antibodies corresponding to mouse-IgG and rabbit polyclonal IgG were tagged with either Fluorescein-IsoThioCyanate (FITC) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) or TetramethylRhodamine-IsoThioCyanate (TRITC®) (Jackson ImmunoResearch Laboratories, Inc.) and incubated simultaneously in PBSt for 1 h at RT (1:100 dilution) in a humidity chamber. In some experiments, multilabeling was performed by including the Hoechst-33258 fluorescent nuclear marker (Invitrogen Molecular Probes, Eugene, Oregon, USA) and Rhodamine-phalloidin a high-affinity F-Actin probe conjugated to the red-orange fluorescent dye, TRITC (Invitrogen Molecular Probes).

For C6-glia cells transfected with GFP-Tau441, fixation was carried out in 100% methanol at -20°C for 5 min and incubated with a pair-wise combination of a monoclonal antibody to α -Tubulin and one rabbit polyclonal antibody to GEF-H1 (Gene-Tex, Inc., Irvine, CA, USA) (see Table 1). Cyanine-5 (Cy5)-tagged anti-mouse IgG and TRITC-tagged anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc.) were employed as secondary antibodies (1:100). These cells were also counterstained with

Table 1
Antibodies and fluorescent markers employed

Antibody	Epitope	Host-class	Procedure	Dilution	Source of reference
Tau-5	210–230**	mouse-IgG	IC, WB	1 : 500	[76]
Tau	Tau	polyclonal rabbit-IgG	IC	1 : 1000	GeneTex, Inc., Irvine, CA, USA
α -Tubulin	α -Tubulin	mouse-IgG	IC	1 : 500	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Truncated-PARP	Asp ²¹⁴ -truncation	polyclonal rabbit-IgG	WB	1 : 1000	Cell Signaling Technology, Danvers, MA, USA
α -Actin	α -Actin	polyclonal rabbit-IgG	WB	1 : 500	[77]
GEF-H1	GEF-H1	polyclonal rabbit-IgG	IC	1 : 500	GeneTex, Inc.
GAPDH	GAPDH	mouse-IgG	WB	1 : 10000	Millipore, Tamecula, CA, USA
Organelle	Fluorescent marker	Excitation/emission (nm)	Procedure	Dilution	Source of reference
Nuclei	Hoechst-33258	(350-461)	FL	1 : 2000	Invitrogen Molecular Probes, Eugene, OR, USA
F-Actin cytoskeleton	Rhodamine-phalloidin	(540-565)	FL	1 : 500	Invitrogen Molecular Probes

IC, immunocytochemistry; WB, western blotting; FL, fluorescence. **Residues based on the longest human Tau isoform (2N4R).

Hoechst-33258 to visualize the nuclei. In some experiments, Tau-expressing C6-glia cells were incubated with RhoA, ROCK, and Myosin II ATPase activity inhibitors (C3-transferase, Y-27632, and Blebbistatin, respectively) (Cytoskeleton Inc., Denver, CO, USA; Tocris Bioscience, Bristol, UK and Sigma Aldrich, respectively) [37, 38] and, based on Tau, F-Actin, Tubulin, and nuclei staining, some changes in cytoskeletal and PM organization were analyzed. Immunolabeled cells were commonly analyzed by epifluorescence through a 20X (Numerical aperture [NA]: 0.5) and a 40X (NA: 0.75) Nikon Plan-Fluor lens coupled to a Nikon Eclipse-80i Microscope (Nikon Corp., Tokyo, Japan). The images were obtained and collected by using a Nikon digital sight-DG-Ri1 camera controlled with the NIS-Elements AR-3.0-SP7 software included in the system (Nikon Corp.). Quantitative counting of fluorescent cells was conducted at low magnification in recorded images. For multiple labeling, optical sectioning, colocalization, and critical observations of subcellular components, images were collected in a TCS-SP8 confocal laser-scanning microscope by using a 63X (NA: 1.4) OIL PH3 CS2 HC PL APO lens (Leica Microsystems, Heidelberg, Germany).

Analysis of cells extracts by electrophoresis and western blotting

After cell transfection and treatments with some drugs, the cell cultures were washed twice with PBS, scraped and lysed in Radio-Immuno-Precipitation-Assay (RIPA) buffer containing a cocktail of proteases inhibitors (150 mM NaCl, 50 mM TRIS, pH 8.0, 1% Triton X-100, 0.5% sodium deoxycholate,

1 mM PMSF, 100 mM NaF, 1 mM Na₃VO₄, and 2 μ g/mL Complete; Roche, Indianapolis, IN, USA) and centrifuged for 10 min at 12,000 g. In some experiments, non-transfected cells were previously treated with either 1 μ M staurosporine (STS) (Sigma-Aldrich) or 1 μ g/mL Actinomycin-D (Act-D) for 12 h to induce apoptosis, and then lysed and processed in the same manner. The supernatant was collected and the protein content in the samples was determined by means of the Mini-Bradford assay utilizing the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Inc.). Total extracts of the cells containing 30 μ g of protein were mixed in 2X sample buffer (TRIS-HCL 100 mM pH 6.8, sodium dodecyl sulfate (SDS) 4%, bromophenol blue 0.2%, β -mercaptoethanol 5%, and glycerol 20%) and boiled at 95°C for 5 min. Proteins from the samples were separated by electrophoresis on 8–10% SDS-polyacrylamide gel (SDS-PAGE) and then transferred onto a nitrocellulose membrane for immunoblotting analysis. Membranes were blocked in 5% nonfat dried milk in PBS-tw (PBS-0.1% Tween 20) overnight at 4°C. All primary antibodies (see Table 1) were diluted in PBS-tw and incubated at RT for 1 h. After washing in PBS-tw, incubation with the corresponding peroxidase-conjugated secondary antibody to either mouse or rabbit (1 : 10,000; ZYMED Invitrogen, Carlsbad, CA, USA) was carried out for 1 h in PBS-tw. Bands of immunoreactive proteins were visualized after membrane incubation in Western Lightning[®] Plus-ECL Enhanced Chemiluminescence Substrate (PerkinElmer, Inc., Waltham, MA, USA) and developed on autoradiography films (Kodak Medical X-ray, general-purpose-blue; Eastman Kodak Co., Rochester, NY, USA) according to the manufacturer's instructions.

TUNEL assay

To determine whether Tau affects the survival of C6-cells, the Terminal deoxynucleotidyl transferase-mediated dUTP Nick End Labeling (TUNEL) was performed by using *In Situ* Cell Death Detection Kit (ROCHE Applied Science, Mannheim, Germany). Briefly, cells were transfected with the empty vector and incubated for 12 h in the presence of STS (1 μ M) to induce chemical apoptosis. The cells were fixed with 4% formaldehyde for 1 h at RT, rinsed twice in PBS, and permeabilized with 0.1% Triton X-100 in freshly prepared 0.1% sodium citrate for 2 min on ice. After rinsed twice in PBS, the coverslips were incubated with TUNEL reaction mixture for 15 min at 37°C, and then incubated with Tau 46.1 antibody to detect Tau. The coverslips were rinsed with PBS and incubated with Hoechst-33258 for 5 min and mounted with Vectashield.

Scratch-wound assay

C6-glia cells were grown to confluence on 15.6 mm culture dishes, transfected for 48 h and treated for 2 h with 12 μ M mitomycin C to inhibit proliferation during the experiment. Cell cultures were scratch-wounded using a sterile 10 μ l pipette tip, washed twice with PBS and re-fed with DMEM-F12 with 10% FBS. After 48 h, C6-cells were fixed and the progress of cell migration into the wounded area was observed by immunofluorescence with antibodies to Tau (polyclonal) and α -Tubulin (mouse monoclonal).

RESULTS

Transient expression of Tau alters the normal morphology and the Tubulin cytoskeleton of C6-glia cells

Tau expression was induced in C6-glia cells to evaluate alterations in cell morphology and the disturbance of the intracellular organization of the cytoskeleton. The expression of either Tau441 or Tau421, which is a proteolytic product of Caspase-3 action [17], was confirmed by immunofluorescence (Fig. 1A, B) and immunoblot analysis (Fig. 1D) in 48 h post- and non-transfected C6-glia cells (transfection efficiency around 40%). A polyclonal antibody to either variant of Tau, in combination with a monoclonal antibody to α -Tubulin, was utilized for immunofluorescence analysis. C6 cells transfected with the empty vector were not recognized by the

polyclonal antibody to Tau (left panel, Fig. 1A), which indicates the absence of endogenous expression of the Tau in these glial cells. Immunoblot analysis with the Tau-5 antibody, which recognizes residues 210–230 in the molecule of Tau (see Table 1) also confirmed the lack of endogenous expression of Tau in both normal cells and in cells only transfected with the empty vector (pcDNA3.1) (Fig. 1D). For extracts of cells expressing either Tau441 or Tau421, Tau-5 recognizes the corresponding bands of 65 kDa and 63 kDa, respectively. Expression of Tau441 and Tau421 was also corroborated by using Tau-46.1 and Tau-C3 antibodies, which specifically recognize the intact C-terminus and Asp⁴²¹-truncation in the molecule, respectively (data not shown).

In culture, C6-glia cells display a normal spindle-like morphology with radiating distribution of microtubules along the cytoplasm (white arrows, Fig. 1A, B). In contrast, when C6 cells expressed either Tau441 or Tau421, severe morphological changes were observed, such as the formation of PM blebs along the entire cell surface (yellow arrows, Fig. 1A, B). In the majority of Tau-expressing cells, morphological change was also accompanied by abnormal bundling of the Tubulin cytoskeleton (arrowheads, Fig. 1A, B). These microtubule bundles were commonly observed in the vicinity of the nuclear region (arrowheads, Fig. 1B), which we previously reported as also occurring in Tau-transfected SH-SY5Y cells [18]. We found that 84% (Standard deviation [SD], $\pm 1.5\%$) of Tau-expressing cells develop PM blebs (Fig. 1C). Likewise, when the number of cells exhibiting PM blebs was compared and analyzed by Student *t* test, no difference was found between Tau441-expressing or Tau421-expressing cells ($p = 0.4641$; Not significant [NS]).

When Tau-expressing cells were viewed through differential interference contrast (DIC) microscopy, the manner in which these cells acquired deformed multilobular morphology was noticeable (arrows, Fig. 2A), in comparison with the flat shape displayed by non-transfected cells. The lack of induced apoptosis in Tau-expressing cells was evidenced by negative staining with the TUNEL assay (Fig. 2B, C). After 48 h of transfection, cell viability was evaluated by using the MTT assay and expression of either Tau441 or Tau421 in C6-glia cells did not decrease this parameter when compared with empty vector-transfected cells ($p = 0.803$ by one-way Analysis of variance [ANOVA]) (Fig. 2D). Even after 72 h of transfection, no difference was found between these groups.

Fig. 1. Altered phenotype of Tau-expressing C6-glia cells. Full-length Tau (Tau441) and Asp⁴²¹-truncated Tau (Tau421) were transiently expressed in C6-glia cells. In A, images represent the merge channels of double immunolabeling assay for Tau variants (green) and Tubulin (red). Immunofluorescence was conducted with a rabbit polyclonal antibody to Tau and a monoclonal antibody to α -Tubulin (see Table 1). pcDNA3.1: empty vector. Panel B displays individual channels where the same combination of antibodies was utilized. In the merge channel, nuclear labeling with Hoechst-33258 was also included. Normal Tubulin cytoskeleton in non-transfected cells is indicated with white arrows. Note the formation of pericentric rings of microtubules (arrowheads) and PM blebs (yellow arrows) in Tau-expressing cells. This abnormal phenotype was observed in 84% of Tau441-expressing cells (affected cells in C). D) Western blotting analysis with Tau-5 antibody (see Table 1) confirms the expression of both Tau441 and Tau421 variants of Tau, and the absence of these proteins in control and non-transfected C6-glia cells. Scale bars: 15 μ m in A, and 10 μ m in B.

Fig. 2. PM blebbing of Tau-expressing cells is not associated with apoptosis. A) Differential interference contrast (DIC) microscopy allows visualizing the change of shape and volume of Tau441-expressing cells. Immunolabeling with Tau-5 confirms the expression of Tau in C6-glia cells undergoing PM blebbing (arrows). C6-glia cells only transfected with the empty vector were pretreated with 1 μ M staurosporine (STS) and analyzed for the development of apoptotic nuclei with the TUNEL assay and immunolabeled with Tau-46.1 antibody (donated by Dr. V. Lee) to the C-terminus of Tau (B). Apoptotic nuclei are evident (arrows). In C6-glia cells expressing Tau (positive to Tau-46.1) but not treated with STS, TUNEL assay was negative (C). In both conditions, nuclei were counterstained with Hoescht-33258 (blue color in merge). D) MTT assay demonstrated that no changes on cell viability were produced when Tau-expressing cells and empty vector-transfected cells were compared ($p=0.803$ by one-way ANOVA). E) When non-transfected cells were treated with STS and Act-D (1 μ g/mL), the apoptotic PARP (Asp²¹⁴) cleavage product was significantly generated, but this was not the case for non-treated C6 cells expressing either Tau441 or Tau421. No degradation of Actin was observed in total extracts from Tau441- and Tau421-expressing cells. Scale bar in A: 10 μ m; in B and C: 15 μ m.

To further discard the possibility that PM blebs formation was the result of Tau-induced apoptosis, we evaluated the formation of proteolytic products of the activity of the Caspases, such as Asp²¹⁴-cleaved Poly (ADP)-ribose polymerase (PARP) in Tau-expressing and non-transfected C6 cells.

By employing a specific antibody to Asp²¹⁴-cleaved PARP in western blotting (WB) analysis of cells extracts, only a faint band corresponding to this product was observed in both Tau441 and Tau421-expressing cell extracts (upper panel, Fig. 2E). This product is less detected in non-transfected cells. For comparative purpose, an increased band corresponding to Asp²¹⁴-cleaved PARP is observed in non-transfected cells induced to apoptosis with either STS (1 μ M) or Act-D (1 μ g/mL) (last two rows, upper panel, Fig. 2E). The expression level of Actin was not altered under any condition (lower panel, Fig. 2E). These results indicate that PM blebs do not originate as apoptotic consequences in Tau-expressing cells.

Plasma membrane blebbing in Tau-transfected C6-glial cells depends on F-Actin remodeling

Because the Actin cytoskeleton has been involved in dynamic changes of the PM in several types of cells [39], we evaluated whether this fibrillary component was affected in Tau-expressing C6-glial cells. For this purpose, these cells were processed for immunofluorescence with the polyclonal antibody to Tau in combination with Rhodamine-phalloidin, a specific fluorescent marker of F-Actin [40]. As illustrated in Fig. 3A, cells not expressing Tau display spindle-like morphology evidenced by a cortical array of the Actin cytoskeleton and Actin-conformed stress fibers (arrowheads). In contrast, in both Tau441- and Tau421-expressing cells, strong PM blebbing is produced in close association with changes in F-Actin distribution (red channel, Fig. 3B, C). Under this condition, Actin-stress fibers disappeared and the cortical pool of F-Actin now surrounded the large number of blebs emerging from the PM (arrows, Fig. 3B, C). It appears that Tau-induced remodeling of the Actin cytoskeleton is involved in PM blebbing. To evaluate this possibility, we treated Tau-expressing cells with 20 μ M Cytochalasin-D (a depolymerization agent of F-Actin) for 30 min, and both the integrity of this component and the formation of PM blebs were analyzed by immunofluorescence.

As presented in Fig. 3D, when Tau441-expressing cells were incubated with Cytochalasin-D, formation of PM blebs was prevented (arrow in green

channel, Fig. 3D) in close association with disruption of the F-Actin cytoskeleton (red channel, Fig. 3D). In the majority of cells, only a positive pool of Actin remains in polarized clusters (yellow arrow, Fig. 3D). To further validate the contribution of the F-Actin cytoskeleton to the formation of PM blebs, Cytochalasin-D was washed away from treated Tau-expressing cells, and 30 min later; the Actin cytoskeleton was reorganized in close association with the formation of PM blebs (yellow arrows, Fig. 3E). In non-transfected cells, the Actin cytoskeleton is restored in normal cortical distribution (asterisk, Fig. 3E). Similar results were observed when Tau421-expressing cells were treated and washed with this drug (data not shown). Significant differences among Cytochalasin-D-treated, Cytochalasin-D-treated and washed, and non-treated C6 cells are depicted in Fig. 4A.

To further analyze the contribution of both Tubulin and Actin cytoskeleton components to PM blebbing, C6-glial cells not expressing Tau (Fig. 5) were treated with 10 μ M Taxol, a microtubule polymerization agent, to mimic the Tau-induced microtubule bundling effect. Taxol treatment for 3 h promoted the formation of thick microtubule bundles (arrows, Fig. 5B), but not the formation of PM blebs. The F-Actin cytoskeleton remains unaltered in these cells, with normal distribution along the cell cortex (asterisk, Fig. 5B). For comparative purpose, the normal cytoskeleton in empty-vector transfected cells is depicted in Fig. 5A.

In contrast, when the normal microtubule network was disrupted in these cells due to incubation with 5 μ M Nocodazole, PM blebbing, and changes in cortical F-Actin organization were also produced (arrows, Fig. 5C); likewise, this was observed in Tau-expressing cells (arrows, Fig. 5D). Quantitative analysis of these effects is shown in Fig. 4B. These results may indicate that abnormal bundling of microtubules specifically induced by Tau promotes a reassembly of the F-Actin that gives rise to the formation of PM blebs.

Tau-induced plasma membrane blebbing is associated with activation of the Rho-GTPase-ROCK pathway

In different eukaryotic cells, stabilization of the microtubule lattice is a regulated process that depends on the binding of several microtubule-associated proteins (MAPs) [41]; however, additional molecules may also bind to these structures with a double

Fig. 3. Tau-induced PM blebbing is caused by remodeling of the F-Actin cytoskeleton. In all panels, Tau441 and Tau421 were recognized with a polyclonal antibody to Tau (green) and the F-Actin cytoskeleton with Rhodamine-phalloidin (red). Nuclei stained with Hoechst-33258 are included in the merge channel (light blue). Note the clear remodeling of F-Actin cytoskeleton in close association with the formation of cortical blebs in both Tau441- (B) and Tau421- (C) expressing C6-glia cells (arrows). F-Actin depolymerization with Cytochalasin-D (20 μ M) rescues the normal phenotype of C6-glia cells, with less formation of PM blebs (arrow in D). When Cytochalasin-D is washed from the cells, both F-Actin remodeling and PM blebbing are reverted in Tau-expressing cells (yellow arrows in E). In A, for comparison, C6 cells are transfected with the empty vector and a normal F-Actin cytoskeleton is depicted (arrowheads). Scale bars: 20 μ m in A; 8 μ m in B; 9 μ m in C, and 15 μ m in D and E.

Fig. 4. PM blebbing in Tau441-expressing C6-glia cells is affected by drugs that interfere with the organization and remodeling of F-Actin and Tubulin cytoskeletons. A) The number of Tau-expressing C6-glia cells that developed PM blebs is reduced to 5% when Cytochalasin-D (20 μ M) is incubated in the growth media for 30 min. After 30 min of Cytochalasin-D washing, approximately 85% of the cells expressing Tau441 again came to develop PM blebs. B) Non-Tau-expressing cells were independently incubated with Taxol (10 μ M) or Nocodazole (5 μ M), and the induction on PM blebs was compared to that produced by Tau-expressing cells. Nocodazole- but not Taxol-treatments produced PM blebs in more than 80% of the cells analyzed. C) When C6 cells expressing Tau441 were incubated with either C3-transferase (0.5 μ g/mL) (to inhibit RhoA-GTPase activity), Y-27632 (10 μ M) (to inhibit ROCK), or Blebbistatin (10 μ M) (to inhibit Myosin-II activity), the number of cells showing PM blebs was significantly reduced in comparison to non-treated cells. * p <0.0001, by one-way Analysis of variance (ANOVA) and consecutive Tukey's multiple comparison test.

purpose as follows: i) contribution to microtubule stabilization, and ii) transient immobilization for self-inactivation. GEF-H1 is one of these molecules that, in turn, activates a small GTPase referred to as RhoA [42]. Bound to microtubules, GEF-H1 is inactivated; however, when the microtubule depolymerizes, GEF-H1 is released into the cytoplasm, wherein it activates GTPase-RhoA in a downstream sequence. Because GEF-H1 is a relevant factor for microtubule stabilization, we evaluated the expression and distribution of this factor in Tau-expressing C6-glia cells showing PM blebs.

As depicted in Fig. 6A, in C6 cells with no Tau expression, the majority of the GEF-H1 is observed in close association with the microtubule lattice

(arrows). Bound to microtubules, GEF-H1 immunostaining appears to emulate a fibrillary appearance. In contrast, when Tau-expressing cells were analyzed under the same immunostaining protocol, the majority of GEF-H1 labeling is observed in association with thick rings of microtubule bundles (arrowheads, Fig. 6B); however, some amount of immunodecorated GEF-H1 also remains free in the cytoplasm and is distributed to the PM blebbing area (arrows, Fig. 6B). The latest effect was not produced by Taxol-induced microtubule bundling, and the entire pool of GEFH-1 remains bound to these polymerized structures (arrows in Fig. 6C-D).

Because these results indicate that some amount of GEF-H1 is displaced from the microtubule

Fig. 5. In non-Tau-expressing C6-glia cells, Taxol induced the formation of microtubule bundle, but not PM blebbing. In all panels, Tubulin and F-Actin were detected with a monoclonal antibody to α -Tubulin and Rhodamine-phalloidin, respectively. In panels A-C, nuclei were identified by Hoechst-33258 staining (in yellow pseudocolor). Normal morphology of C6-glia cells is displayed in panel A. When non-Tau-expressing C6 cells were incubated with Taxol (10 μ M), Tubulin bundling was observed (arrows in B), but neither F-Actin remodeling nor PM blebbing were visualized (asterisk). In contrast, when cells were treated with Nocodazole (5 μ M), microtubules were depolymerized and both F-Actin remodeling and PM blebbing were also evidenced (arrows, in C). D) In comparison, Tau-induced microtubule bundling in C6-glia cells leads to the formation of PM blebs and F-Actin remodeling (arrows). Tau441 was visualized with a rabbit polyclonal antibody to Tau (see Table 1). Scale bars: 20 μ m in A; 7 μ m in C, and 13 μ m in B and D.

lattice to the cytoplasm under Tau expression, this Rho exchange factor might in turn catalytically activate distinct Rho GTPases, such as RhoA [43], which participates in a catalytic pathway that regulates membrane-Actin cortex interactions for cell motility [44–48]. To probe this, we assessed the role of

RhoA in the formation of PM blebs in Tau-expressing cells by incubating these with the exoenzyme C3-transferase, an ADP-Ribosyl Transferase (ADPRT) that selectively ribosylates Rho proteins in the effector-binding domain on asparagine⁴¹, rendering them inactive [37]. In C6 cells with no expression

Fig. 6. In C6-glia cells, expression of Tau produces some release of GEF-H1 from microtubules into the cytoplasmic space. A) In non-Tau-expressing cells, GEF-H1 (recognized with a rabbit polyclonal antibody to GEF-H1) is closely associated with microtubules (recognized with a monoclonal antibody to α -Tubulin). The nucleus stained with Hoechst-33258 is included in the merge channel. B) When Green fluorescent protein-fused Tau (GFP-Tau441) is expressed, triple co-localization is observed among Tau, GEF-H1, and polymeric Tubulin (arrowheads); however, some amount of GEF-H1 labeling is also present in the cytoplasmic space (arrows). No evidence of free cytoplasmic GEF-H1 is found when the microtubule bundling effect was induced with Taxol in cells with no expression of Tau (C: empty vector transfected cells; D: non-transfected cells [NTc]). Most of the labeling with anti GEF-H1 colocalizes with polymeric α -Tubulin in these cells (see merge in C and D). Arrows in C and D indicate strong sites of colocalization. Scale bars: 10 μ m in A and C, 3 μ m in B, 15 μ m in D.

of Tau, incubation with C3-transferase (0.5 μ g/mL) for 48 h did not show any alteration in the normal organization of the cytoskeleton components and cell morphology (Fig. 7A). In comparison with the membrane blebbing formation observed in Tau-expressing cells (Fig. 7B), when C3-transferase was incubated

while Tau expression began in a separate group of transfected cells, no evidence of PM blebbing was observed during 48 h of treatment (green channel, Fig. 7C). Concomitantly in these C3-transferase-treated C6 cells, the F-Actin cytoskeleton did not show any cortical remodeling associated with the

Fig. 7. PM blebbing and F-Actin remodeling in Tau-expressing C6-glia cells are regulated by the RhoA-GTPase-ROCK pathway. In all panels, Tubulin, F-Actin, and Tau441 were detected with a monoclonal antibody to α -Tubulin, Rhodamine-phalloidin, and a rabbit polyclonal to Tau, respectively. In the merge channel, nuclear staining with Hoechst-33258 is included. In A, non-Tau-expressing C6 cells were incubated with C3-transferase (0.5 μ g/mL), a RhoA inhibitor, and no morphological changes are observed. In B, Tau441-expressing cells show PM blebbing, F-Actin remodeling, and microtubule bundling. C) When these cells were treated with C3-transferase, the abnormal phenotype showing PM blebs associated with F-Actin remodeling was avoided. Similarly, this abnormal phenotype was prevented when Tau441-expressing C6 cells were incubated with either 10 μ M Y-27632, an inhibitor of ROCK (D), or 10 μ M Blebbistatin, a Myosin-II inhibitor (E). Under incubation with these inhibitors, the microtubule bundling induced by Tau was not altered and notably, it is more evident how the F-Actin cytoskeleton reorganizes according to the pattern displayed by microtubules (arrows, in C). Scale bars: 10 μ m in A, D and E; 6 μ m in B, and 11 μ m in C.

formation of PM blebs (red channel, Fig. 7C). Instead, this cytoskeletal component appears to adopt a new structure that is closely associated with microtubule bundles (arrows, Fig. 7C).

Because this result indicates that activated-RhoA participates in F-Actin and PM remodeling in Tau-expressing cells, we evaluated whether this GTPase acts through the downstream effector ROCK to increase PM blebbing. To accomplish this, while Tau expression began, transfected-C6 cells were co-incubated with Y-27632 ((+)- (R)-trans-4- (1-aminoethyl)-N- (4-pyridyl) cyclohexanecarboxamide+++dihydrochloride) (10 μ M), a widely used specific ROCK inhibitor [38], and both F-Actin remodeling and PM blebbing were evaluated and compared with non-treated Tau-expressing C6 cells.

In accordance with Fig. 7A, in cells with no expression of Tau, no changes in either F-Actin organization or in the general morphology of the cells were visualized during 48 h of incubation with Y-27632 (not shown). In contrast, both the PM blebbing and F-Actin remodeling observed in Tau-expressing cells (Fig. 7B) were reverted when Y-27632 was included in the incubation media (arrows, Fig. 7D). Note that cells with no expression of Tau are unaffected by this treatment, and both Tubulin and F-Actin cytoskeletons remain unaltered (asterisks, Fig. 7D). Under either C3-transferase or Y-27632 treatments, the Tau-promoted microtubule bundling effect is not disturbed (see blue channel, Fig. 7C, D), which emphasizes the specificity of these drugs in regulating F-Actin remodeling. In control experiments, the rescued effects of both C3-transferase and Y-27632 in avoiding PM blebbing and F-Actin remodeling were not sustained for long when these drugs were washed from the cells, thus reestablishing the abnormal phenotype produced by the expression of Tau (not shown). Quantitative data significantly representing the effect of the previously mentioned inhibitors are presented in Fig. 4C. Because Actin-myosin interactions may take place in the Tau-induced PM blebbing, Tau-expressing C6 cells were incubated with Blebbistatin (10 μ M), a non-muscle Myosin-II-specific inhibitor [49], to validate the participation of Myosin-II in this process. As it was expected, the Tau-induced PM blebbing was avoided (arrow, Fig. 7E), likewise the cortical distribution of F-Actin mediating the formation of PM blebs (Fig. 7E). Quantitative data representing the significance of this effect is presented in Fig. 4C.

All of the previously mentioned results indicate that PM blebbing and F-Actin cytoskeleton

remodeling are produced by Tau expression through activation of the GEF-H1-RhoA-ROCK pathway.

To give evidence for physiological alterations of the abnormal phenotype observed in C6-glial cells expressing Tau, we evaluated their migration *in vitro* by using a scratch-wounded assay. As shown in Fig. 8A, after stimulation with high concentrations of FBS, significant amount of empty-vector-transfected cells migrate to the wounded area to occupy the available space (arrowheads). By contrast, when Tau441-expressing cells were also stimulated, only few amounts of these cells migrate to the wounded area (arrows). Quantitatively, less than 20% of the cells that express Tau migrated to this area. This result indicates that Tau-induced abnormalities in the cytoskeleton and PM, are also accompanied by physiological alteration in the motility of C6 glial cells.

DISCUSSION

In the present study, we sought evidence to better understand whether accumulation of Tau in glial cells, as it occurs in several tauopathies, produces alterations in cell functioning, as has been reported for neurons.

The major aim was to evaluate whether full-length Tau and its Asp⁴²¹-truncated variant induce alterations in cell morphology, particularly in the cytoskeleton and PM integrity, when transiently expressed in C6-glial cells. Our results indicated that both Tau variants produced strong PM blebbing and F-Actin cytoskeleton remodeling. We focused on some intracellular mechanisms leading to F-Actin remodeling, and found that it is produced by indirect activation of the GEF-H1-RhoA-ROCK pathway. All of these actions were initiated by abnormal, induced MT bundling and cytoplasmic release of GEF-H1.

We found that neither full-length Tau nor Asp⁴²¹-truncated Tau produced significant cell death when expressed in glial C6 cells (Fig. 2), contrasting with early evidence reporting immediate induction of apoptosis by C-terminus truncated Tau variants [13, 50, 51]. However, disturbance in the organization and functioning of distinct organelles has been attributed to truncated Tau variants as a gain of toxic functions [10, 11, 52] and, as we previously showed, this occurred in neurons [18], in the present study we also corroborated that expression of Asp⁴²¹-truncated and non-truncated Tau produced abnormal bundling of microtubules in C6-glial cells (Fig. 1A, B). In agreement with preceding studies [19, 20, 53], increased

Fig. 8. Tau expressing cells show reduced migration in a scratch-wounded assay. A) 48 h after empty-vector transfected cell cultures were scratch-wounded, C6 cells migrated to the wounded area (WA) (arrowheads). C6 cells were immunolabeled with a monoclonal antibody to α -Tubulin. Yellow lines indicate the initial border of the scratched area. B) When Tau-expressing cell cultures were scratch-wounded, a few amount of these cells migrated to the WA (arrows) during 48 h. Scale bars: 100 μ m.

microtubule bundling in C6-glia cells was the result of the binding of Tau to these structures (Fig. 6B). The biological consequences of this action can be reflected in changes in the distribution and transport of several membranous elements inside the cells [23]. As an illustration of this action, in Tau-expressing rat astrocytes, increased bundling of microtubules was also reported but in addition a decrease in kinesin-dependent trafficking of membranous elements and collapse of the intermediate filament network was also evidenced [34].

On the other hand, the formation of extensive PM blebs in Tau-expressing C6-glia cells (Figs. 1 and 2) is a remarkable finding that has not been commonly observed in distinct cellular contexts, with the exception of one report, in which a few examples of similar structures, referred to as Tau-positive blobs, were only considered sites of peripheral accumulation of Tau [34]. Under the expression of Tau, we evidenced a further formation of PM blebs that should be considered more of a pathological effect altering the normal architecture of C6-glia cells. In this regard, and despite the morphological similarities, it is unlikely that Tau-induced PM blebbing represent the formation of apoptotic bodies, because no evidence of apoptotic markers or changes in cell

survival were found in either Tau-expressing or non-transfected C6-glia cells (Fig. 2). We consider that PM blebbing in glial cells would represent a pathological action that might alter several properties of the membrane, such as permeability, fluidity, signaling, protein localization, and cytoskeletal anchorage, thus severing the global functioning of this cellular type. We demonstrated at least that migration in Tau-expressing C6-glia cells is physiologically compromised. Because of the pathological consequences of this action, in this study we further characterized the mechanism by which Tau induced PM blebbing in C6-glia cells.

PM blebbing in Tau-expressing cells depends on F-Actin cytoskeleton remodeling

Because PM blebbing represented a specific effect produced by Tau expression and due to that microtubule bundles were not localized in association with these blebs, we searched for alterations in the organization of the F-Actin cytoskeleton, which is known to regulate cell shape and dynamics [39]. In astrocytic cells, the Actin cytoskeleton is involved in additional functions, such as calcium mobilization, signaling, glutamate uptake, and the hormone

modulation of cell growth [54–56]. We observed, in C6-glia cells, that the normal F-Actin cytoskeleton is distributed cortically and is localized in stress fibers and focal adhesions that attach the cell surface to the substrate [57]. Because this F-Actin meshwork was dramatically remodeled in Tau-expressing C6 cells and observed in close association with emerging PM blebs (Fig. 3), we conclude that this type of cytoskeleton regulates the formation of these abnormal protrusions of the PM. This statement was corroborated when Tau-expressing C6 cells were incubated with Cytochalasin-D, a drug that depolymerizes the F-Actin cytoskeleton [58], and no formation of PM blebs was observed (Fig. 3D). Moreover, the reversibility by which PM blebs were formed again when Cytochalasin-D was eliminated from the media of the cells clearly demonstrates the regulatory role of F-Actin in conducting the formation of these abnormal structures. This result is in agreement with early evidence proposing that the Actin-myosin system is the driving power that promotes cell contraction and the formation of PM blebs and apoptotic bodies under physiological and pathological conditions in response to different stimuli [59, 60].

Tau-induced PM blebs are formed by activation of RhoA-GTPase and ROCK

PM blebbing is a dynamic process implicated in cytokinesis and cell movement, but also in pathological conditions, such as apoptosis and cancer cell invasion. Several studies have demonstrated that PM blebbing depends on the promotion of F-Actin-myosin interactions, which is regulated by RhoGTPase-ROCK signaling [44–47]. In our study, we corroborated that PM blebbing in Tau-expressing C6 cells was also regulated by RhoA-GTPase and ROCK signaling, because the addition of their specific inhibitors, C3-transferase and Y-27632, respectively, avoided the formation of these structures (Figs. 7C, D). ROCK is a major effector of RhoA-GTPase, and it was initially characterized for its role in mediating the formation of RhoA-induced stress fibers and focal adhesions [61, 62]. ROCK is a serine/threonine kinase that can directly phosphorylate the Myosin light-chain (MLC) or indirectly increase MLC phosphorylation (MLC-pp) by inactivating the myosin phosphatase [63, 64]. In our Tau-expressing C6-glia cells, we would expect that active ROCK would contribute to F-Actin-myosin force generation, causing blebs to protrude through MLC-pp. Confirmation of F-Actin-myosin interaction during Tau-induced PM

blebbing was obtained when cells were incubated with Blebbistatin, a specific inhibitor of Myosin-II activity [49], and neither F-Actin remodeling nor PM blebs formation were observed (Fig. 4C).

Because F-Actin remodeling is involved in the formation of distinct types of PM protrusions, such as lamellipodia and filopodia that depends on the activation of different GTPases, it was difficult to rule out the participation of Rac-1 and Cdc42 in the formation of PM blebs in the C6 cells. However, we discarded this possibility because the inclusion of NSC23766, a specific inhibitor of Rac1 [65], did not affect the formation of PM blebs (data not shown). With these experiments, we emphasize the role of RhoA-GTPase in the regulation the dynamics of F-Actin remodeling associated with the formation of PM blebs that, in our particular case, is not initiated by external stimuli [59, 66, 67]. Instead, this is produced only by the presence of Tau in the cytoplasmic space. This comprises a relevant observation, and we can speculate that, even in the absence of external stimuli, only intracellular accumulation of Tau in glial and neuronal cells occurring in distinct tauopathies may render and initiate pathologic alterations in PM architecture and functioning. Despite the evidence that Tau may interact with Actin under several normal and pathological conditions [25, 28, 31, 32], in our control experiments, we did not find direct interactions among these proteins; however, under incubation with either C3-transferase or Y-27632, a close association between F-Actin and microtubule bundles, probably mediated by Tau, was observed. This result supports recent *in vitro* evidence that Tau can induce guided polymerization of Actin filaments along microtubule tracks [27, 68]. Whether Tau or other cytoskeletal-associated proteins are responsible for this interaction is another interesting issue for further investigation.

GEF-H1: the link between Tau-induced microtubule bundling and F-Actin-dependent PM bleb formation

Despite that F-Actin remodeling through activation of the RhoA-GTPase-ROCK pathway was responsible for PM blebbing formation in C6 cells, precisely how this cascade with no extracellular stimuli was initiated, represented a major issue to be addressed. How could Tau, with its increased microtubule-bundling properties, initiate activation of the RhoA-ROCK-Actin-myosin pathway, which mediates PM bleb formation? Given that RhoA does not exhibit clear co-localization to microtubules [69],

direct interaction between these two participants in activating RhoA is unlikely. However, the participation of additional upstream candidates implicated in the RhoA-ROCK pathway, such as GEF, would address this issue. Of the several classes of GEF, the catalytic activity of GEF-H1 is uniquely regulated by its localization to the microtubules. Depolymerization of microtubules leads to GEF-H1 activation, while relocalization to microtubules inhibits its activity [43]. In this regard, when we analyzed GEF-H1 localization in Tau-expressing C6-glia cells, we found that some of this factor remains bound to the microtubule bundles; however, a free pool of this factor was also observed in the cytoplasm (Fig. 6B). We think that under Tau-expression, the induction of microtubule bundling also leads to the release of the microtubule-bound GEF-H1 into the cytoplasm, which in turn may activate RhoA-GTPase and consequently the previously mentioned F-Actin remodeling pathway. This result confronts the traditional statement that depolymerization of the microtubule lattice is the key factor for releasing GEF-H1 into the cytoplasm. Tau-induced microtubule bundling is a specific process for inducing the previously noted effects, because incubation with Taxol to stimulate the same effect was not sufficient to induce the release of GEF-H1 to the cytoplasmic space (Figs. 5B; 6C-D). Because Tau and GEF-H1

share localization in the microtubule bundles (Fig. 6B), it may be possible that the binding of Tau along the microtubule surface in some way displaces some amount of the microtubule-bound GEF-H1 into the cytoplasm; however, more experiments are required to be conclusive.

Overall, it has been demonstrated that the regulated release of GEF-H1 from microtubules is a recurring mechanism for achieving localized activation of RhoA in several physiological processes [70–72]; however, this regulation is also implicated in the development of numerous pathophysiological conditions, including chronic diarrhea [73], cardiomyopathies [74], and cancer [75]. Tau-induced PM blebbing, responding to changes in the Tubulin and F-Actin cytoskeletons, may then result in a pathological condition that alters the normal functioning of glial cells, thus comprising a relevant factor that contributes to neuronal dysfunction.

In summary, the present study further contributes to the description a new molecular mechanism for Tau-induced neuropathology and degeneration in astrocytes. When expressed in astrocytic C6 cells, Tau toxicity was represented by the formation of abnormal blebs along the PM, associated with remodeling of the Tubulin and F-Actin cytoskeletons. This phenotype was produced by the activation of the RhoA-GTPase-ROCK pathway that regulates

Fig. 9. Proposed mechanism of Tau-induced PM blebbing in glial cells. Increased concentration of Tau in the cytoplasm produces abnormal bundling of microtubules. Although the mechanism by which this occurs remains unknown, microtubule bundling may allow the release of some amount of microtubule-bound GEF-H1 into the cytoplasm, where this nucleotide exchange factor may activate RhoA-GTPase. If so, this GTPase drives the active ROCK to phosphorylate several effectors, including myosin light-chains. This action may promote F-Actin-myosin interactions that lead to remodeling of the F-Actin cytoskeleton and the formation of PM blebs. In AD and other tauopathies, these morphological alterations may take place and contribute to the impairment of glial activity.

F-Actin-myosin interaction. We think that this cascade is initiated upstream by the release of GEF-H1 into the cytoplasm, due to the abnormal bundling of Tubulin promoted by Tau (Fig. 9). Because glial cells contribute to the neuronal maintenance and survival, Tau-induced alterations in these cells may also account for the neurodegenerative process observed in the brains of individuals suffering from tauopathies.

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