Fragmentation of the Golgi Apparatus in Neuroblastoma Cells Is Associated with Tau-Induced Ring-Shaped Microtubule Bundles

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Abstract. Abnormal fibrillary aggregation of tau protein is a pathological condition observed in Alzheimer's disease and other tauopathies; however, the presence and pathological significance of early non-fibrillary aggregates of tau remain under investigation. In cell and animal models expressing normal or modified tau, toxic effects altering the structure and function of several membranous organelles have also been reported in the absence of fibrillary structures; however, how these abnormalities are produced is an issue yet to be addressed. In order to obtain more insights into the mechanisms by which tau may disturb intracellular membranous elements, we transiently overexpressed human full-length tau and several truncated tau variants in cultured neuroblastoma cells. After 48 h of transfection, either full-length or truncated tau forms produced significant fragmentation of the Golgi apparatus (GA) with no changes in cell viability. Noteworthy is that in the majority of cells exhibiting dispersion of the GA, a ring-shaped array of cortical or perinuclear microtubule (Mt) bundles was also generated under the expression of either variant of tau. In contrast, Taxol treatment of non-transfected cells increased the amount of Mt bundles but not sufficiently to produce fragmentation of the GA. Tau-induced ring-shaped Mt bundles appeared to be well-organized and stable structures because they were resistant to Nocodazole post-treatment and displayed a high level of tubulin acetylation. These results further indicate that a mechanical force generated by tau-induced Mt-bundling may be responsible for Golgi fragmentation and that the repeated domain region of tau may be the main promoter of this effect.

Keywords: Alzheimer's disease, confocal microscopy, Golgi apparatus, immunofluorescence, microtubule lattice, tau

INTRODUCTION

In Alzheimer's disease (AD), tau protein undergoes several post-translational modifications, such as phosphorylation [1–3], conformational changes [4–6], and truncation [7–9] that convert this protein into a non-functional entity with more aggregative properties.

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Regarding toxicity, controversy exists concerning aggregated or non-aggregated tau as the more pathologic entity in AD; however, non-fibrillary forms of tau may play a significant role in producing subtle and progressive alterations in organelle structure and functioning [10, 11].

Still under debate, early studies documented the acute toxicity of pathologically truncated forms of tau, which were able to produce apoptotic cell death when expressed within several cellular contexts [12–14]; notwithstanding this, more recent evidence demonstrated that abnormal accumulation of tau, to a greater degree than lethal consequences, produces a series of morphological and organizational alterations in cells that may gradually lead to functional impairments [15–17].

In this regard, when expressed in primary cultured neurons, full-length- and truncated-tau induced mitochondrial fragmentation reduced their calcium-buffered capacity, as well as increased the level of intramitochondrial reactive oxygen species. To some extent, these abnormal effects were associated with tau-promoted activation of calcium-dependent calcineurin phosphatase [15, 18]. Furthermore, truncated fragments corresponding to the N-terminus of tau (26–230 aa) produced functional and structural alterations in the mitochondria when expressed in primary cultured neurons as well [16, 19].

Because abnormalities in membranous organelles such as mitochondria and nuclei were also described by microscopic examination of brain tissue from AD patients [20, 21], we previously investigated *in vitro* whether cytoplasmic overabundance of tau contributes to the morphological disturbance of intracellular organelles in cultured neurons and glial cells.

We focused on the comparative actions produced by truncated and non-truncated forms of tau by the transiently expression of these recombinants in the SH-SY5Y neuroblastoma cells. To the same extent, both Asp⁴²¹-truncated and full-length tau produced extensive deformation of the cellular nuclei [17]. We concluded that more than direct interactions between elements of the nuclear membrane and tau, the nuclear deformation was produced by a constriction force generated by tau-induced cytoplasmic rings of bundled microtubules (Mts). The same expression of these variants of tau in cultured glial C6 cells also produced massive lobulations of the plasma membrane that were not associated with the expression of apoptotic markers [22]. The mechanism underlying this unusual action was characterized, and it was proposed that an abnormal tau-induced Mt-bundling effect

produced the release of the Mt-attached GEF-H1 [23] to the cytoplasm. In doing so, this guanine exchange factor switched on the RhoAGTPase-ROCK pathway which regulates and reorganizes the cortical F-actin cytoskeleton; thus, this activation promoted the formation of abnormal blebs along the plasma membrane.

On the other hand, the functional relevance of the Golgi apparatus (GA) in processing, packing, and sorting newly formed proteins has been largely documented [24–26]. In many types of cells, including neurons, this membranous organelle, structured as stacks of parallel cisternae, is mostly located in the vicinity of the centrosomes and is stabilized by interactions with both centrosomal and GA-derived Mts [27–29]. Multiple associations through some Mt-binding proteins and proteins from the GA matrix, such as γ -tubulin and p115 [30], GM130 [31], CLASP [32], and GMAP-210 [33], allow physical association and interaction between the GA complex and the Mt network.

The structure of the GA is not excluded from being affected in neurons in the brain of AD patients. Morphological studies have reported that this organelle is fragmented and atrophic in different areas of the brain of AD cases and independent of the load of either plaques or neurofibrillary tangle (NFT)-bearing neurons [10, 11, 34]. Recently, by analyzing cortical and hippocampal areas of the brain of AD cases utilizing immunofluorescence and 3-Dimensional (3D)-confocal microscopy, profound and higher levels were found of GA fragmentation in NFT-bearing neurons in comparison with cells devoid of these fibrillary structures [35, 36]. Studies in animal models representing tauopathies have also documented fragmentation of the GA in neurons that carried or did not carry fibrillary structures composed of hyperphosphorylated tau [37, 38].

From all this evidence, it appears likely that abnormal consequences of the persistent redistribution and propensity to self-aggregation of tau into the somatic compartment also comprises alteration in the structure of distinct membranous organelles including the GA.

The mechanism by which tau may alter the organization of the GA in AD is not yet understood; however, several groups have attempted to clarify this issue by analyzing the consequences of exogenous expression of normal or modified tau in cultured neuronal and non-neuronal cells.

In this regard, the overexpression of mutated forms of tau, P301L, V337M, and R406W increased the

capability of the wild-type form to produce GA fragmentation in rat hippocampal cultures [37]. When the longest human tau isoform was expressed in primary cultures of rat astrocytes, several intracytoplasmic alterations were produced, including a reduction of kinesin-dependent intracellular trafficking, decrease of detyrosinated Mts, and remarkable fragmentation of the GA [39].

Direct interaction of exogenously expressed tau with GA membranes was also evidenced in hippocampal cultures from rat embryos and, despite this action can be considered a physiological mechanism, concomitant fragmentation of this membranous organelle may also imply a pathologic consequence [40]. Yet to be determined, several possibilities have been proposed to explain the mechanism by which tau alters GA architecture. Because tau may interact with membranes through its proline-rich domain at the N-terminus [41], a direct association between tau and GA cisternae may affect the stability of the multilamellar complex. Another possibility is that increased amounts of tau may disturb the association and functioning of Golgi-associated structural proteins, such as GRASP55/65, and those from Golgi matrix proteins, including GM130 and Golgin-160 [42].

As mentioned previously, the organization and structure of the GA is mostly dependent on the Mt network and several proteins that mediate their interactions. This dependency has long been corroborated *in vitro* when GA fragmentation was observed in cells treated with several Mt depolymerizing agents [43–45]. However, it remains controversial that, in some reports, chemically induced polymerization of tubulin, also produced GA fragmentation [45, 46]. With respect to tau-induced GA fragmentation, some authors have thought that this alteration may be indirectly related to disturbance in the Mt lattice [39]; however, little evidence concerning this effect as a primary cause of GA dispersion is presented and barely discussed [37], and more experiments need to be conducted to better clarify this issue.

In the present study, we further added evidence about the pathologic effects that tau expression produces in neuroblastoma cells, focusing on the alteration of membranous organelles and the cytoskeleton. We found profound GA dispersion when full-length tau was expressed, with no intensification in this effect under the expression of pathogenic truncated tau variants. It appears likely that the Mt-binding repeated domains present in all tau recombinants analyzed may be responsible for

tau-induced GA fragmentation. Remarkably, in the majority of cells exhibiting GA-dispersion, characteristic ring-shaped Mt bundles were also observed in cortical and perinuclear regions of the cell. By employing drugs that alter the stability of the Mt lattice and of the GA architecture, we characterize the steadiness of these tau-induced Mt bundles and conclude that specific formation of these well-organized structures is likely the main mechanism associated with fragmentation of the GA. As a pathological consequence of this action, a decrease in the carbohydrate content was found in full-length tau-expressing cells.

MATERIALS AND METHODS

Plasmid constructs

Preparation of plasmids encoding full-length tau (Tau441), Asp⁴²¹-truncated tau (Tau421), and GFP (Green Fluorescent Protein)-fused Tau441 (GFP-Tau441) were described previously [17]. In the current study, new primers used to generate plasmids for the expression of additional truncated forms of tau are presented in Table 1.

Plasmids were obtained as polymerase chain reaction (PCR) products utilizing the pcDNA3.1 Zeo(-) full-length tau plasmid as an original template and Taq DNA polymerase (Promega, Madison, WI, USA). PCR products and pcDNA3.1 Zeo(-) plasmid were digested with EcoRI and XhoI endonucleases (New England BioLabs, Inc.) and purified with the GenElute™ kit (Sigma-Aldrich). Finally, tau fragments were ligated to empty pcDNA3.1 Zeo(-) plasmid using the T4 DNA Ligase (New England BioLabs, Inc.). Positive colonies were analyzed by restriction analysis using the previously mentioned endonucleases. The integrity and correct sequence of all truncated tau variants were confirmed by sequence analysis.

SH-SY5Y cell culture and transfection

SH-SY5Y human neuroblastoma cells were acquired from American Type Culture Collection (ATCC) (Manassas, VA, USA) and cultured in DMEM-F12 medium complemented with 10% (v/v) Fetal Bovine Serum (FBS) (Invitrogen Life Technologies-GIBCO, Carlsbad, CA, USA), 100 U/mL Penicillin, 100 µg/mL Streptomycin, and maintained under a humidified atmosphere of 5% CO₂ at 37°C. Culture medium was replaced every 2 days.

Table 1
Primers used to prepare plasmids for Tau-truncated fragments

Truncated forms of Tau	Forward	Reverse
Tau1-391 (Glu ³⁹¹ -truncated Tau)	5'- CGGCTCGAGATGGCTGAGCCCCGCCAGGAG-3'	5'- CCGGAATTCCGGTTACTCCGCCCCGTGGTC-3'
Tau150-441 (Tau-Ile ¹⁵⁰ -Leu ⁴⁴¹)	5'- CGGCTCGAGATGAAGATCGCCACACCGCGG-3'	5'- CCGGAATTCTTACAAACCCTGCTTGCCAG-3'
Tau123-391 (Tau-Thr ¹²³ -Glu ³⁹¹)	5'-CGGCTCGAGATGACCCAAGCTCGCATGGTC-3'	5'- CCGGAATTCCGGTTACTCCGCCCCGTGGTC-3'

When neuroblastoma cells reached 70–80% confluence, they were transiently transfected with the plasmid pcDNA3.1 Zeo(-), which contained the sequences for either Tau441, Tau421, Tau1-391, Tau150-441, or Tau123-391, utilizing Lipofectamine 2000 and following the manufacturer's instructions (Invitrogen Life Technologies-GIBCO). The same experimental conditions were employed for transient transfection of the plasmid that contains the sequence for either GFP-Tau441 or GFP-alone (pVLGT42). The efficiency of transfection for each plasmid is reported in Supplementary Figure 1.

Twenty-four to 48 h after transfection, neuroblastoma cells were washed in Phosphate Buffered Saline (PBS) solution pH 7.4 and processed for several experimental procedures.

Additional transfections with plasmids pmTurquoise2-Golgi, which was donated by Dorus Gadella (Addgene plasmid # 36205), and mCherry-Tubulin-C18, provided by Michel Davidson (Addgene plasmid # 55148), were conducted to visualize the GA and tubulin cytoskeleton under fluorescence microscopy, respectively [47, 48].

Cell viability

Tau-expressing and control non-transfected SH-SY5Y cells were evaluated for viability by using the MTT (3-(4, 5-diMethylThiazol-2-yl)-2, 5-diphenyl Tetrazolium bromide) (Sigma-Aldrich, St. Louis, MO, USA) assay [49]. In brief, cells were plated in ELISA (Enzyme-Linked ImmunoSorbent Assay) plates and washed with fresh culture medium. Thereafter, cells were incubated with MTT (0.5 mg/mL) prepared in fresh medium for 3 h at 37°C. After washing, cells were then incubated with DMSO (DiMethyl SulfOxide) (Sigma-Aldrich) to dissolve the formazan aggregates. Finally, the intensity of the colored MTT products was read at 570 nm employing

an ELISA microplate reader (iMark; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Electrophoresis and western blot analysis of cell extracts

Transfected and non-transfected neuroblastoma cells were washed with ice-cold PBS twice, scraped, lysed in Radio-Immuno-Precipitation-Assay (RIPA) buffer that contained a cocktail of proteases inhibitors (150 mM NaCl, 50 mM Tris, pH 8.0, 1.0% Triton X-100, 0.5% sodium deoxycholate, 1 mM PMSF, 100 mM NaF, 1 mM Na₃VO₄, 2 µg/mL Complete; Roche, Indianapolis, IN, USA) and then centrifuged for 10 min at 12,000 g. Pellets were discharged, the supernatant was collected, and the protein concentration was determined by Mini-Bradford assay utilizing the Bio-Rad protein assay reagent (Bio-Rad). The samples containing 30 µg of protein were diluted 1:1 in 2X sample buffer (Tris-HCL 100 mM pH 6.8, SDS 4%, bromophenol blue 0.2%, β-mercapto-ethanol 5%, and glycerol 20%) and boiled at 95°C during 5 min. These samples were subjected to electrophoresis on 8% Sodium Dodecyl Sulfate-PolyAcrylamide Gel (SDS-PAGE) and then transferred onto a nitrocellulose membrane for immunoblotting analysis. Blocking of membranes was performed by incubation in 5% non-fat-dried milk in PBS-t (PBS-0.1% Tween 20) overnight at 4°C. Primary antibodies (see Table 2) were diluted at the corresponding concentration in PBS-t and incubated at room temperature (RT) for 1 h. Membranes were washed with PBS-t, and then incubated with the corresponding peroxidase-conjugated secondary antibody to either mouse or rabbit (1:20,000; ZYMED Invitrogen, Carlsbad, CA, USA) for 1 h at RT in PBS-t. Bands of immunoreactive proteins were visualized after membrane incubation in Western Lightning Plus-Enhanced ChemiLuminescence

Fig. 1. Full-length Tau variants expressed in SH-SY-5Y cells. A) Schematic representation of Tau constructs: (I) the longest isoform (441 amino acids); (II) GFP-tagged longest isoform (441 amino acids). Exons 2 and 3 (E2,E3), Proline-rich region (P1,P2), Repeated domains 1–4 (R1–R4). B) Western blot analysis of cell extracts obtained 48 h post-transfection. Tau-5 and a rabbit polyclonal antibody to Tau (see Table 2) recognize extracts of Tau441- and Tau441-GFP-expressing cells. A goat polyclonal antibody to GFP (Table 2) identifies GFP- and Tau441-GFP-expressing cells. Non-transfected (control) and empty-pcDNA-transfected cells were negative to these antibodies. Tubulin recognized by a mouse-monoclonal antibody to α -tubulin (see Table 2) was used as a loading control. C) Tau441-GFP-expressing cells were fixed in 0.3% glutaraldehyde-PBS and processed for immunofluorescence by using the anti- α -tubulin antibody. Arrows in confocal microscopy images denote ring-shaped microtubule (Mt) bundles caused by the expression of Tau441-GFP. Asterisks indicate non-transfected cells with normal tubulin cytoskeleton. Nuclear blue staining observed in the merge channels corresponds to fluorescent Hoechst-33258. Scale bars in all panels: 20 μ m.

Table 2
Antibodies and fluorescent markers employed

Antibody	Epitope	Host-class	Procedure	Dilution	Reference
Tau-5	210–230**	mouse-IgG	IC, WB	1:500, 1:10,000	Provided by L I. Binder
Tau-13	9–18**	mouse-IgG	WB	1:10,000	[4]
Tau 46.1	428–441**	mouse-IgG	WB	1:10,000	Provided by Virginia Lee
α -Tubulin	α -Tubulin	mouse-IgG	IC	1:500	Santa Cruz Biotechnology
Acetyl- α -Tubulin (Lys-40)	Acetyl- α -Tubulin	rabbit-IgG	IC	1:200	[56]
GAPDH	GAPDH	mouse-IgG	WB	1:10,000	Millipore
RCAS 1	Gly 147	rabbit-IgG	IC	1:100	Cell Signaling
Polyclonal to Tau	Center region	Rabbit-IgG	WB	1:10,000	GeneTex
Polyclonal to GFP	Internal region of GFP	Goat-IgG	WB	1:10,000	Santa Cruz Biotechnology
Monoclonal to Golgin-97 (CDF4)	Human Golgin-97	mouse-IgG	WB	1:1000	Molecular Probes
Organelle	Fluorescent marker	Excitation/emission (nm)	Procedure	Dilution	Reference
Nuclei	Hoechst-33258	350/461	FL	1:2,000	Invitrogen molecular probes
F-Actin cytoskeleton	Rhodamine- phalloidin	540/565	FL	1:500	Invitrogen

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

substrate (ECL) (Perkin Elmer, Inc., Waltham, MA, USA) and developed in autoradiography films (Kodak Medical X-ray, General Purpose/Blue) (Eastman Kodak Company, Rochester, NY, USA) according the manufacturer's instructions.

Determination of glycoprotein and carbohydrate content

Carbohydrate content of transfected and non-transfected neuroblastoma cells was assessed by using a glycoprotein carbohydrate estimation kit (Thermo Fisher Scientific, Waltham, MA, USA). By following manufacturer's instructions, PBS-washed cells were extracted and prepared in the glycoprotein assay buffer at 2.5 mg/mL. Negative (Lysozyme) and positive (Ovalbumin, Apo-Transferrin, Fetuin, and α -Acid Glycoprotein) standard samples were also plated and analyzed in parallel. After chemical reaction with the glycoprotein-detection reagent, absorbance values at 550 nm were used to estimate the carbohydrate content of cell samples and for comparison with the standards of the known carbohydrate content [50].

Immunofluorescence and confocal microscopy

Transfected and non-transfected cells, treated or not with some drugs, were processed for

multilabeling immunofluorescence. Initially, cells were fixed with 2% paraformaldehyde at RT for 15 min and permeabilized in 0.1% Triton X-100-PBS. Afterward, cells were blocked in a solution containing 0.5% gelatin and 1.5% FBS in PBS for 1 h at RT. Single- or double- labeling with primary antibodies (Table 2) was conducted for 1 h at RT in a humidity chamber. Pair-wise combinations of antibodies were carefully selected to avoid crosstalk and non-specific cross-reactions. Secondary antibodies corresponding to mouse-IgGs and rabbit polyclonal IgGs were tagged with either Fluorescein-IsoThioCyanate (FITC) (Jackson Immuno-Research Laboratories, Inc., West Grove, PA, USA) or TetramethylRhodamine-IsoThioCyanate (TRITC) (Jackson Immuno-Research), and incubated simultaneously in PBS-t for 1 h at RT. In some experiments, triple-labeling was conducted by including the Hoechst-33258 fluorescent nuclear marker (Invitrogen- Molecular Probes, Eugene, OR, USA). Filamentous actin (F-actin) was evaluated in some cells by counterstaining with Rhodamine-Phalloidin (Rh-Ph), a fluorescent phallotoxin with specific avidity for F-actin, but not for the soluble pool of unpolymerized actin molecules [51].

When SH-SY5Y cells expressed GFP-Tau441, fixation was first carried out in 0.3% glutaraldehyde for 3 min at RT, permeabilized with 0.1% Triton X-100-PBS, and then post-fixed in 0.3% glutaraldehyde in

PBS for another 10 min. After washing, cells were post-treated with sodium borohydride (10 mg/mL in PBS) for 7 min and incubated with 0.01 M glycine in PBS for 20 min. Next, these cells were incubated with a monoclonal antibody to α -tubulin (1:500) and/or one polyclonal antibody to the GA (Receptor binding Cancer Antigen expressed on SiSo Cells) [RCAS1] (Table 2). TRITC-tagged anti-rabbit IgGs and Cyanine-5 (Cy5)-tagged anti-mouse IgGs (Jackson Immuno-Research) were utilized as secondary antibodies (1:500). In some experiments a rabbit monoclonal antibody to Acetyl- α -Tubulin (Lys40) (Cell Signaling, Danvers MA, USA) was also included in the procedure.

Immunofluorescent cells were viewed and analyzed by epifluorescence through a 20X (Numerical Aperture [NA]: 0.5) and a 40X (NA: 0.75) Plan-Fluor Lens coupled with a Nikon Eclipse-80i Microscope (Nikon Corp., Tokyo, Japan). Images were obtained and recorded by using a Nikon digital sight-DG-Ri1 camera controlled with the Nikon NIS-Elements AR-3.0- SP7 software included in the system (Nikon). Quantitative counting of fluorescent cells was conducted at low magnification. For multiple labeling, optical sectioning, colocalization, and critical observations of subcellular components, images were acquired by confocal microscopy using a TCP-SP8 confocal laser scanning microscope coupled with a 63X (NA: 1.4) OIL PH3 CS2 HC PL APO lens (Leica Microsystems, Heidelberg, Germany). Quantitative colocalization was evaluated by using the colocalization tool included in the LAS-X software (Leica SP8 TCS-SP8). Significance of colocalization was validated by Pearson's correlation analysis [52].

In some images, area and perimeter of the GA were obtained to calculate the index of compactness according to the following principle: $4\pi \text{ area} / \Sigma \text{ perimeter}^2$ [53, 54].

RESULTS

Overexpression of full-length- and truncated-tau variants in SH-SY5Y cells produces fragmentation of the Golgi apparatus

To further investigate alterations in the organization of intracellular compartments produced by cytoplasmic overabundance of tau protein, we transiently transfected undifferentiated SH-SY5Y cells with plasmids encoding the sequence of either full-length tau (Tau441) or full-length tau attached to GFP (Tau-GFP) (Fig. 1A). After 48 h of transfection,

independent Tau441 and Tau441-GFP expression was demonstrated by western blot analysis of total cell extracts with either Tau-5 (Fig. 1B), which recognize the 210–230 amino acid sequences in the molecule, or a generic rabbit-polyclonal antibody to the center region of this protein (Table 2, Fig. 1B). Tau expression was observed in Tau441- (~65 kDa) and Tau-GFP- transfected cells (>75 kDa), but not in neuroblastoma cells only transfected with either the empty vector (pcDNA3.1), the GFP- codifying vector (GFP), or non-transfected control cells (Fig. 1B).

Selective expression of GFP and Tau441-GFP was also demonstrated by the use of a goat polyclonal antibody to GFP (Table 2) (Fig. 1B). As we have reported previously, under our cell culture conditions, expression of endogenous tau is inappreciable (Supplementary Figure 2).

When these cells were processed for immunofluorescence with a monoclonal antibody to α -tubulin, the majority of the cells undergoing Tau441 expression (GFP-attached) also developed abnormal Mt bundles organized as cortical rings (arrows in Fig. 1C), which are not observed in non-transfected cells (asterisks in Fig. 1C). In parallel, when other groups of tau-expressing cells were also transfected with a plasmid encoding for fluorescent Turquoise2-beta-1,4-galactosyltransferase-1 to visualize the GA, we observed profound fragmentation of this organelle in the same cells (arrows in Fig. 2B). This effect was closely associated with the formation of the above mentioned tau-induced Mt bundles (arrowheads in Fig. 2B), because in control cells only expressing GFP (Fig. 2A), neither ring-shaped Mt bundles, nor GA fragmentation were observed. In these cells with no expression of tau, the morphology of this organelle is compact and predominantly located in the juxtannuclear area (asterisks in Fig. 2A). As we have previously described [17], some tau-expressing cells exhibit a more polyhedral morphology and increase in size. At least for 48–72 h of transfection, the previously mentioned effects in tau-expressing cells were not significant in terms of producing cell death, as indicated by the MTT assay (Supplementary Figure 1).

To corroborate the effect of tau in producing GA fragmentation, we also evaluated GA organization by immuno-detection with a polyclonal antibody to RCAS1, a type-III transmembrane GA protein [55]. As shown in Fig. 2C, cells expressing Tau441 (arrows) also exhibited ring-shaped Mt bundles (arrows) and GA fragmentation (arrowheads). In these cells, the compressed morphology of this

Fig. 2. Full-length Tau expression produces fragmentation of the Golgi apparatus. SH-SY5Y cells expressing either GFP-alone (A) or Tau441-GFP (B) were co-transfected with the plasmid pmTurquoise2-Golgi to visualize the morphology of the Golgi apparatus (GA), and immunolabeled for tubulin cytoskeleton. C) Tau441-GFP-expressing cells were double labeled with a rabbit polyclonal antibody to the Receptor binding Cancer Antigen expressed on SiSo Cells (RCAS1), and the mouse-monoclonal antibody to α -tubulin. While arrows indicate fragmentation of the GA in B, asterisks in A denotes the compact organization of the same structure. Arrowheads in B, also indicate the formation of ring-shaped Mt bundles in Tau-expressing cells. Scale bars in all panels: 20 μ m.

organelle is lost and resembles a fragmented structure with multiple vesicles spreading to the cytoplasmic space (arrowheads in Fig. 2C). Cells with no expression of tau again demonstrated no alteration in GA morphology (asterisks in Fig. 2C). Quantitative determination of GA compactness corroborated a significant reduction in this parameter in tau-expressing cells when compared to non-transfected cells (Supplementary Figure 6A).

In view of the fact that acetylation of tubulin is considered a post-translational modification that confers more stability to Mts [56], we evaluated the occurrence of this modification in the tubulin cytoskeleton of transfected and non-transfected cells. As shown in Fig. 3A and B, the level of tubulin acetylation was higher in tau-induced ring-shaped Mt bundles (arrows) than in those Mts observed in either non-transfected cells or cells with low expression of tau (asterisks). Noteworthy that GA dispersion (observed by Turquoise2-Golgi expression) is confirmed in tau-expressing cells forming acetylated rings of Mts (arrowheads in Fig. 3A, B). These results were also confirmed by PAGE and western blot analysis of cell extracts obtained from both control and tau-expressing cells. As shown in supplementary Fig. 3A, a significant increase of acetylated tubulin is found in tau-expressing cells, but not in control cells with no expression of tau.

Because tau truncation has been considered an abnormal modification that may increase its pathologic effects in AD, we evaluated whether C-terminus truncation of tau at either Asp⁴²¹ (Tau421) or at Glu³⁹¹ (Tau391) potentiate the previously mentioned GA fragmentation. SH-SY5Y cells were independently transfected with plasmids encoding for these truncated variants, but preserving intact their N-terminus (Fig. 4AII, 4AIII). Congruently, specific expression of these truncated molecules was corroborated by PAGE and western blot analysis of total extracts from tau-expressing cells (Fig. 4B, C). Likewise, specific bands for either Tau421 or Tau391 were confirmed with Tau-5 (Fig. 4B) and Tau-13 antibodies (Fig. 4C).

When these cells were analyzed by immunofluorescence, similarly both truncated tau variants also produced abnormal bundling of the Mt lattice (arrows in Fig. 5). Concomitantly, GA fragmentation was also observed in these cells that independently expressed Tau421 or Tau391 (asterisks in Fig. 5). Again, non-transfected cells displayed the normal packed organization of this membranous organelle (arrowheads in Fig. 5).

Fragmentation of the Golgi apparatus is also produced by N-terminus truncated tau

Because the latter truncated tau variants contained an intact N-terminus, we further investigated whether this portion of the molecule contributed to the previously observed alteration of the GA. For this purpose, we prepared two more truncated tau variants: one consisting of a molecule without the N-terminus but maintaining the proline-rich region and the C-terminus (Tau150-441) intact, and the other variant, which was truncated at both N- and C- termini (Tau123-391) (Fig. 6AIV, AV).

Specific expression of either Tau150-441 or Tau123-391 was corroborated by western blot analysis of total cell extracts from transfected cells (Fig. 6B). While Tau-5 antibody recognized both truncated proteins, Tau-46.1 only detected that preserving its C-terminus (Tau150-441).

As depicted in Fig. 6C, cells undergoing expression of either Tau150-441 or Tau123-391, clearly exhibited GA fragmentation (arrows) in comparison with the normal and compact appearance of this organelle in cells lacking tau expression (asterisks). Expression of Tau123-391 was also capable of inducing highly acetylated ring-shaped Mt rings (Supplementary Figure 3A,B). Quantitatively, no truncated-forms of tau increased the percentage of cells undergoing GA dispersion in comparison with those expressing the full-length tau (Tau441) (Fig. 6D) ($p=0.52$, by one way-analysis of variance [ANOVA]).

Fragmentation of the Golgi apparatus is produced by drug-induced destabilization of the microtubule lattice

Because tau-induced tubulin bundling appeared to be associated with resulting GA fragmentation, we tested the normal stability of the Mt network in non-transfected cells and evaluated its consequences on the normal morphology of this membranous organelle. In Fig. 7Aa, normal morphology of the GA and the tubulin lattice is presented. However, as previously described, Nocodazole treatment (5 μ M) disrupted normal Mt lattice organization and caused extensive GA fragmentation (arrows in Fig. 7Ab). In contrast, when cells were incubated with 10 μ M of Taxol (a drug that promotes Mt polymerization) to mimic tau-induced Mt-bundling, the majority of the cells did not show GA dispersion (asterisks in Fig. 7Ac). As a comparative effect on GA fragmentation, a group of non-transfected cells was treated

Fig. 3. Acetylation of tubulin is increased in Tau-induced ring-shaped Mt bundles. In A and B, Tau-GFP-expressing cells were co-transfected with pmTurquoise2-Golgi, and multi-labeled for total tubulin (red channel), Acetylated-tubulin (Ac-Tubulin) (rabbit monoclonal antibody to Acetyl- α -Tubulin (Lys40), and nuclei (Hoechst-33258). Arrows indicate highly acetylated microtubules (Mts). Asterisks point to cells with reduced level of tubulin acetylation. Fragmentation of the GA is indicated with arrowheads. Scale bars: 20 μ m in A; 7 μ m in B.

independently with BreFeldin-A (BFA), a drug that interferes with anterograde transport from the endoplasmic reticulum to the GA, but that also disrupts

the stacking organization of the GA lamellae [57]. As illustrated in Fig. 7Ad, in BFA treated cells, Mt lattice organization is retained as normal (arrowheads),

Fig. 4. Truncated-Tau variants expressed in SH-SY-5Y cells. A) Schematic representation of Tau constructs: (I) the longest isoform (441 amino acids); (II) Asp⁴²¹-truncated-Tau (Tau421); (III) Glu³⁹¹-truncated-Tau (Tau391). B, C) Western blot analysis of cell extracts obtained 48 h post-transfection. Tau-5 (B) and Tau-13 (C) antibodies (see Table 2) recognize extracts of Tau441-, Tau421-, and Tau391-expressing cells. Non-transfected (control) and empty-pcDNA-transfected cells were negative. Mouse anti-GAPDH (see Table 2) was used as loading control.

but GA organization is collapsed into dispersed vesicles yet is localized around the juxtannuclear area (arrows). Quantitative data supporting the previously mentioned effects are represented in Fig. 7B.

All these results may indicate that normal GA architecture depends on a balanced amount and the specific organization of Mts inside the cells, which

in turn can be altered by the overabundance of tau protein. Remarkably, overexpression of mCherry-Tubulin in neuroblastoma cells was unable to induce ring-shaped Mt bundles such as those produced by the overabundance of tau (Supplementary Figure 4).

To further investigate the tau-induced force mediating Mt-bundling, cells were treated with Taxol to

Fig. 5. C-terminus truncated Tau produces Mt-bundling and GA dispersion. Both Tau421- and Tau391-expressing cells developed ring-shaped Mt bundles (arrows) and GA fragmentation (asterisks). Normal morphology of the GA is indicated with arrowheads. A, B) Rabbit polyclonal antibody to Tau and mouse monoclonal antibody to α -tubulin. C, D) Tau-5 antibody (generic marker of Tau; see Table 2) and rabbit polyclonal antibody to RCAS1. In merge, nuclei staining with Hoechst-33258 is included. Scale bars in all panels: 20 μ m.

stabilize the Mt network initially, and then Tau441 was expressed to evaluate possible alterations in the organization of this component. As shown in Fig. 8A

and B, 30-min incubation with Taxol preceding tau expression was not sufficient to avoid the formation of ring-shaped Mt bundles (arrows). Nevertheless,

Fig. 6. N-terminus-truncated Tau causes GA fragmentation. A) Schematic representation of Tau constructs: (I) the longest isoform (441 amino acids); (IV) N-terminus truncated-Tau (Tau-Ile¹⁵⁰-Leu⁴⁴¹); (v) N- and C-termini-truncated Tau (Tau-Thr¹²³-Glu³⁹¹). B) Western blot analysis of cell extracts from non-expressing and Tau-expressing neuroblastoma cells. While Tau-5 antibody recognizes all the variants of Tau, Tau-46.1 (a C-terminus marker of Tau; Table 2) only detects those that maintain an intact C-terminus (Tau441 and Tau150-441). GAPDH is used as loading marker (mouse monoclonal antibody to GAPDH; see Table 2). C) Both Tau150-441- and Tau123-391-expressing cells were immunolabeled with Tau-5 and rabbit polyclonal anti RCAS1. Nuclei were labeled with Hoechst-33258. Arrows indicate cells expressing N-terminus-truncated Tau developing GA dispersion. Asterisks denotes non-transfected cells with normal and compact morphology of the GA. D) Graphic representation of normalized values of Tau-expressing cells depicting GA dispersion. No significant differences among the groups were found by one-way ANOVA ($p = 0.52$). Scale bar in all panels: 20 μ m.

Fig. 7. Chemically-induced disturbance of the microtubule lattice alters the structure of the Golgi apparatus. Non-transfected cells were treated with distinct drugs that alter either tubulin lattice (Nocodazole and Taxol), GA structure (Brefeldin-A), or F-actin cytoskeleton (Cytochalasin-D). Only Nocodazole (panel Ab) and Brefeldin-A (panel Ad) produced fragmentation of the GA, verified by immunolabeling with previously described antibodies to RCAS1 (arrows) and α -tubulin. Brefeldin-A did not alter the normal tubulin lattice (arrowheads in panel Ad). Asterisks denote normal compact GA morphology (panels Aa and Ac). B) Graphic representation of normalized values of treated-cells undergoing GA fragmentation. Significant differences among the groups were found by one-way ANOVA ($p < 0.0001$), and subsequent Tukey's multiple comparison test ($p < 0.001$) for specific pairwise combinations: Control/Nocodazole; Control/Brefeldin-A; Nocodazole/Taxol; Nocodazole/Cytochalasin-D; Taxol/Brefeldin-A; Brefeldin-A/Cytochalasin-D. Scale bar in all panels: 20 μ m.

in many cases, these rings were partially formed and lateral strands of unincorporated Mts were also observed (arrowheads in Fig. 8B). In contrast, when cells already expressing Tau441 were subjected to Nocodazole treatment, the tubulin cytoskeleton was disrupted in the majority of the cells (asterisks in Fig. 8C, D); however, at different degree, ring-shaped Mt bundles yet were observed in those cells that expressed Tau441 (arrows in Fig. 8C, D).

Organization of the Golgi apparatus in SH-SY5Y cells is independent to alterations induced on the actin cytoskeleton

Because we previously reported that tau protein may indirectly alter the organization of membranous components through remodeling of F-actin cytoskeleton [22], we sought evidence to know whether these filamentous elements participate in GA organization and stability. To accomplish this, non-transfected SH-SY5Y cells were incubated with 20 μ M of Cytochalasin-D for 30 min to depolymerize the F-actin cytoskeleton [58]. As presented in Fig. 9B, while F-actin organization (evaluated with Ph-Rh) was disrupted under this condition (asterisks), GA structure remained unaltered (arrows). Quantification of the reduced number of Cytochalasin-D-treated cells undergoing GA fragmentation is demonstrated and compared with other treatments in Fig. 7B.

To further corroborate this observation, in other experiments, cells were independently incubated with two well-known inhibitors of RhoA and Rac-1 GTPases (C3-Transferase and NSC23766, respectively), components of the transduction pathway that regulates F-actin organization and motility in different cell contexts [59, 60]. Under incubation with these compounds, no alteration in GA morphology was observed (arrows in Fig. 9C, D), in that it was comparable with that shown in non-treated cells (arrow in Fig. 9A).

Moreover, in tau-expressing cells where GA dispersion was observed (arrows in Fig. 10), no signs of F-actin alterations were observed (asterisks in Fig. 10). These results indicate that GA organization in undifferentiated SH-SY5Y cells is independent of the structuration of the F-actin cytoskeleton.

Reduced level of Golgin-97 and total carbohydrate content in cells expressing tau

To evaluate whether tau-induced GA dispersion may also alter the expression of constituent proteins

of the GA, the level of Golgin-97, a peripheral membrane protein localized on the cytoplasmic face of the GA [61], was assessed in cells transfected with plasmids encoding for both full-length- and truncated-tau variants. As shown in supplementary Fig. 5, western blot analysis reveals a significant reduction in the level of Golgin-97 in all tau-expressing groups when compared to controls.

Moreover, to support evidence for a functional alteration of cells undergoing tau-induced GA dispersion, estimation of carbohydrate content in cell extracts was conducted by using a Glycoprotein carbohydrate detection kit (Thermo Fisher Scientific, USA). As depicted in Table 3, a significant reduction of a normalized amount of carbohydrates was found in samples corresponding to tau-expressing cells, compared to those of empty-plasmid- or GFP-expressing cells.

DISCUSSION

Intracellular aggregation of tau protein in the form of paired helical filaments is considered one of the major pathologic causes of neuron death occurring in the brain of patients suffering AD and other tauopathies [62, 63]. However, a body of evidence currently supports the fact that oligomeric non-fibrillary aggregates of tau may be also implicated in primary toxicity in these neurons [64–66].

Moreover, by using *in vitro* cultured cells that exogenously expressed either native or modified tau, several investigation groups have confirmed the induction of abnormalities in cell structure and organelle functioning [67–71]. In primary immortalized cultured neurons, the expression of Asp⁴²¹-truncated tau altered mitochondrial morphology and function [15, 72]. Notably, not only C-terminus truncated tau can be potentially harmful for the cells: for instance, N-terminus truncation has been also reported [73] and, despite being less characterized, some pathological and regulatory features have been also associated with the occurrence of this modification in AD [73] and within cells contexts [16, 19, 74–77].

By considering the idea that tau protein is capable of altering the organization of diverse membranous organelles, as reported for the nuclear compartment in neurons of AD patients [21, 78], we sought additional cytotoxic effects produced by the *in vitro* expression of native and Asp⁴²¹-truncated tau in undifferentiated SH-SY5Y neuroblastoma cells. We found that

Fig. 8. Tau-induced ring-shaped microtubule bundles are resistant to agents that destabilize the tubulin cytoskeleton. Neuroblastoma cells were treated with Taxol to stabilize the microtubule lattice initially, and then transfected to express Tau-GFP (A, B). After 48 h of transfection, cells were immunolabeled to visualize the tubulin cytoskeleton. Panel A corresponds to merge channels displaying Tau-GFP, tubulin (Tub), and nuclei labeling. Notice the formation of ring-shaped Mt bundles (arrows). In panel B, lateral strands of Mts (arrowheads) are projecting from a ring-shaped Mt bundle (arrows). When Tau-GFP-expressing cells were post-treated with Nocodazole (C, D), considerable number of ring-shaped Mt bundles (arrows) were resistant to the depolymerizing effect of this drug. In those cells with no expression of Tau, the microtubule lattice is highly disturbed (asterisks in C and D). Scale bars: 20 μ m, 20 μ m, and 15 μ m, from the left to the right in A; 15 μ m in B; 20 μ m in C; and 20 μ m in D.

Fig. 9. In SH-SY5Y neuroblastoma cells, organization of the Golgi apparatus is independent of the structuring of the actin cytoskeleton. Non-transfected cells were treated with drugs that alter or regulate the organization of actin cytoskeleton and then analyzed by double labeling with anti-RCAS1 antibody, and Rhodamine-Phalloidin (Rh-Ph) to visualize Filamentous actin (F-actin). A) Non-treated control cells; B) Cytochalasin treated cells; C) NSC23766 (Rac-GTPase inhibitor) treated cells; D) C3-transferase (RhoA-GTPase inhibitor) treated cells. Arrows in A-D, indicate the normal appearance of the GA. Asterisks in B denote disassembled F-actin cytoskeleton. Scale bars in all panels: 20 μ m.

Fig. 10. Tau-induced Golgi apparatus dispersion is independent of F-actin organization. A, B) Tau-GFP-expressing cells were co-transfected with pmTurquoise2-Golgi and analyzed for the organization of F-actin with Rh-Ph staining. Arrows in A and B indicate GA fragmentation in cells expressing Tau-GFP. In the same cells, F-actin organization is not altered (asterisks in A and B). Scale bars in all panels: 20 μ m.

both variants of tau produced significant deformity of the nuclear compartment, with extensive lobulations along the nuclear envelope [17]. This action was produced by a constraining force generated by perinuclear rings of Mt bundles, which were in turn produced by tau expression.

The GA is another important membranous component involved in the proper post-translational protein processing, trafficking, and sorting [26].

Table 3
Determination of glycoprotein and carbohydrate content

Samples		Carbohydrate content (%), $\pm$ SD
1	Control non-transfected	28 $\pm$ 0.57
2	pcDNA3.1	27 $\pm$ 0.57
3	GFP- alone	27.5 $\pm$ 0.5
4	Tau441	21 $\pm$ 1.52
5	Tau123-391	21 $\pm$ 1.32
6	Tau-GFP	20 $\pm$ 0.76

Normalized values were obtained from high glycosylated proteins used as a reference, $\pm$ SD: Standard Deviation. Significant differences by one-way ANOVA ($p < 0.001$) and subsequent Tukey's analysis were found between Tau-expressing cells (4,5,6) and controls (1,2,3) ($p < 0.005$).

In AD and other neurodegenerative disorders, evidence demonstrating alterations in the GA morphology was obtained from histopathological analysis of brain tissue [36, 79]; and remarkably, fragmentation of this organelle comprises a common feature observed in several of these pathologies. This may indicate that a broad spectrum of intracellular factors and pathologic inducers occurring in the neurons of distinct neurological disorders may, at some point, converge in affecting GA organization and function. Despite that GA fragmentation has been reported in the brain of AD patients for some time [11, 34, 35, 80–82], the precise mechanisms by which this alteration is produced and its possible connection with the intracellular deposition of both fibrillary and non-fibrillary aggregates of tau protein remains under profound investigation.

In the present study, we sought evidence to better understand the molecular mechanisms underlying alterations GA morphology under the cellular expression of truncated tau forms that have been proposed as pathologic in AD and in several cellular contexts. As shown in Figs. 2, 3, 5, and 6, both full-length tau and all variants of truncated tau consistently produced GA fragmentation.

We did not find differences in the percentage of cells undergoing GA dispersion when we expressed and compared full-length tau *versus* distinct truncated variants (Fig. 6D), which may be indicative that the extreme C-terminus of tau is not involving in causing this abnormal effect. Fragmentation of neuronal GA was also observed in rat hippocampal cultured cells that overexpressed full-length human tau, with a significant increment in this effect when such cells expressed either P301L, V337M, or R406W, the mutated forms of tau representative of some tauopathies [37]. Furthermore, when primary hippocampal neurons from rat embryos were transfected

to express the 3-Repeats (3R) isoform of human tau, the same effect of GA fragmentation was observed and, remarkably, in these cells tau was found in close association with GA membranes [40].

From this evidence, it appears likely that the overabundance of tau may be a causative factor in GA dispersion, but the precise mechanisms underlying this action are still elusive. One possibility is that tau may interfere with and disturb the organization and function of some proteins that contribute not only to the stability of the GA complex, such as GRASP65 [83] and Grasp55 [84], but that also interfere with and disturb those involved in trafficking across the Golgi stack, such as GM130 [85], p115 [86], and Golgin-160 [87]. Supporting this idea, we report here that at least expression of Golgin-97, an integral protein of the GA, is decreased in full-length- and truncated-tau-expressing cells (Supplementary Figure 5).

It is noteworthy that in our study, together with the induction of GA dispersion in tau-expressing cells, we also observed the development of abnormal Mt bundles, organized as cortical and pericentral rings (Figs. 2–5). We think that these structures are directly responsible for GA dispersion because, in the same tau-expressing cells, we had already seen that ring-shaped tau-induced Mt bundles produced deformation of the nuclear membrane [17]. Furthermore, the expression of tau and some truncated tau variants in C6-glia cells not only led to the formation of abnormal Mt bundles, we also saw that these structures promoted the development of abnormal lobulations in the plasma membrane [22]. All the evidence indicates that indirectly tau produces alteration in the organization of intracellular membranous compartments as a pathologic mechanism involving the development of specific ring-shaped Mt bundles and changes in cellular tensesity.

It has been largely accepted that, in many cell types, the Mt network and specific GA-associated Mts are important for maintaining GA organization and stability [28, 88]. Consequently disruption of the tubulin cytoskeleton with Mt-destabilizing agents such as Colchicine or Nocodazole, were effectively shown to produce concomitant GA dispersion [44, 89].

Because dispersion of the GA produced by tau-induced Mt-bundling observed in our study contrasted with the same fragmentation of the GA produced by chemical disruption of the Mt lattice, we further analyzed and compared the effect of Taxol, an Mt-bundling promoter, on GA structure. As depicted in Fig. 7Ac, albeit that Mt bundles were produced by Taxol treatment, these bundles were not able to

induce GA dispersion. Moreover, these Mt bundles were not organized as peri-centric rings as were those produced by the expression of tau. As another way to promote Mt-bundling, overexpression of tubulin in control cells did not result in changes in the Mt lattice (Supplementary Figure 4), which further suggest a unique role of tau in forming these pathologic structures. High quantitative colocalization between tau and ring-shaped Mt bundles supports this affirmation (Supplementary Figure 6B).

In additional experiments analyzing the ductility of these structures by incubating tau-expressing cells with Nocodazole, we continued to observe the remains of these ring-shaped Mt bundles in a certain amount of cells (Fig. 8C, D). Contrariwise, in some cells with Mts previously stabilized with Taxol, tau expression was still capable of forming primordial structures resembling Mt rings (Fig. 8A, B). These results demonstrated the steadiness of the Mt-formed structures generated by the cytoplasmic overabundance of tau.

Despite some evidence reporting that Taxol treatment may alter GA organization [46], we think that ring-shaped Mt bundles may comprise a particular and organized structure that is produced only by the specific interaction of tau with Mt. In a recent report on NIH 3T3 fibroblasts, the expression of FHDC1, a formin family protein with Mt-binding capability, produced GA dispersion [90]. However, the only expression of its Mt-binding domain that is more capable of stabilizing Mts [91] was not able to produce GA dispersion.

In our tau-expressing SH-SY5Y cell model, none of the N- and C-truncated forms of tau were able to strengthen the GA dispersion effect observed with full-length tau, which may be indicative that the region of Mt-binding domains, which is common in all tau recombinants employed in our study, is mostly responsible for the ring-shaped Mt-bundling effect. This can be confirmed in future research by evaluating the effects of recombinants either lacking in or carrying modified Mt-binding repeated domains.

In previous studies, the occurrence of tau expression and GA dispersion were reported, however few explanations regarding the mechanisms underlying this effect preceded our interpretations. We further emphasize the contribution of Mt-bundling in producing GA fragmentation. For instance, in cultured rat hippocampal neurons overexpressing either human 3R-tau, or mutated variants of tau representing tauopathies, GA dispersion was reported but not clearly associated with ring-shaped Mts [37, 40]. By

analyzing normal interactions between endogenous tau and GA membranes, it is concluded that tau may directly interact with components of the GA membrane, which also may mediate their associations with the Mt lattice [40].

In another report, the expression of human full-length tau in the primary culture of rat astrocytes produced alteration in several components, including localization and expression of kinesin, Matrix Golgi protein-160 (MG160), intermediate filaments, GA dispersion and, remarkably detyrosinated Mts [39]. Because detyrosinated Mts (Glu-microtubules), to a greater degree than tyrosinated Mts, are more stable structures [92], it is uncertain how tau produced such a reduction in astrocytic cells [39]. Contrariwise, in our study conducted in tau-expressing SH-SY5Y cells, we propose that tau expression produced more stable Mts, which were not only resistant to Nocodazole treatment, but that also displayed higher level of acetylation (Supplementary Figure 3) [56].

It is also noticeable in our study that, under different experimental conditions (Fig. 7), the morphological aspect of dispersed GA vesicles could have been associated with different mechanisms. BF-A treatment, which is known to induce GA dispersion by fusing rough endoplasmic reticulum and GA membranes [57], produced a moderate effect and, despite that multiple vesicles were formed in general, these remained localized in the surroundings of the centrosomal region (Fig. 7Ad). Because tau-induced GA dispersion appeared to exceed the dispersion effect observed with BFA, again we think that this alteration was produced by strong remodeling of the Mt lattice, which may include changes in both cytoplasmic and Golgi-associated Mts [28].

Fragmentation of the GA may compromise several intracellular mechanisms mediated by this organelle such as protein processing and glycosylation, as well as protein sorting and secretion [24–26]. By first time we report that tau-induced dispersion of the GA also produces a decrease in the glycoprotein and carbohydrate content (Table 3), which may have a pathological consequence in protein processing as it was mentioned already.

On the other hand, regardless of the little evidence available, the role of the actin cytoskeleton in maintaining GA architecture and function has been also documented.

In cultured NIH 3T3 fibroblasts, it was demonstrated that GA structural stability depended on the dynamics of actin and the Mts, which were regulated by a common formin protein referred to as FHDC1

[90]. In addition to that FHDC1 has Mt-binding properties, it also possesses an FH2 domain with affinity for the actin cytoskeleton. In the same regard, in HeLa JW cells, GA dispersion and rearrangements of the actin cytoskeleton were observed when the Rho-mDia1 pathway was activated in these cells [54].

Our results are not in line with this evidence, in that the expression of tau in neuroblastoma cells did not produce alteration on the normal organization of F-actin (Fig. 10). This result is reinforced by the inoperability of drugs that either destabilize F-actin organization (Figs. 7B and 9B) [58] or inactivate Rho-GTPases (C3 transferase) [59] to produce GA dispersion (Fig. 9D). These results may indicate that the GA dispersion produced by tau is independent of the organization of the F-actin cytoskeleton in neuroblastoma cells. It is plausible that the contribution of F-actin cytoskeleton in GA architecture depends on the cellular type.

At this point, with this study, we have now further added morphological and structural evidence regarding that the formation of ring-shaped Mt bundles by the overabundance of tau in the cytoplasm is a critical mechanism that produces dispersion of the GA. Because non-fibrillary aggregates of tau are accumulated initially in AD brains, the excess of tau may abnormally contribute to Mt remodeling, GA dispersion, and alteration of protein processing and sorting.

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SUPPLEMENTARY MATERIAL

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